Investigation of oxygen targets and hypoxic adaptation in paediatric critical illness

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Declaration

I, Sainath Raman, confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated within the thesis.

Acknowledgements

I would like to express my sincere gratitude to my primary supervisor Prof M Peters and my secondary supervisor Prof S Rahman. I have new found respect for my wife for supporting me through this endeavour. My proofreaders: Dr Samiran Ray and Dr Aparna Raman spent hours trawling through my thesis and painstakingly corrected it. For this, I will be ever indebted to them.

Contents

Abstract

Background

The effect of varying oxygen tension on the outcome of critically ill patients is contentious. Many interventions employed in the intensive care unit aimed at increasing oxygen delivery can cause harm. During the natural history of critical illness, adaption to tissue hypoxia may occur to a greater or lesser extent. I used the principle of Near Infrared Spectroscopy (NIRS) with a vascular occlusion test to investigate forearm muscle oxygen consumption.

Methods

The relationship between arterial oxygenation and mortality in critically ill children was explored with a retrospective observational study, a national survey and

a systematic review in chapter 3.

Chapter 4 describes a study of Near infrared spectroscopy with a vascular occlusion test (NIRS VOT) in children with suspected mitochondrial disease. The aim was ascertain if NIRS VOT as a technique is able to reflect tissue oxygen consumption.

Chapter 5 covers the Young Everest Study 2. Healthy volunteers (8-16 years) who travelled to Nepal and trekked to 3525 meters altitude were tested at two altitudes (sea level and 3525 meters). The aim was to investigate the physiological response to a hypobaric hypoxic environment including the NIRS VOT.

Chapter 6 covers studies conducted on critically ill children admitted to the PICU in GOSH. These include serial NIRS VOT study, an *in-vivo* measure of oxygen consumption of peripheral blood mononuclear cells and the expression of oxidative phosphorylation (OXPHOS) genes changes in the first 48 hours after admission to the PICU in children with meningococcal septic shock.

Results

Chapter 3

The epidemiological study of the children admitted to the intensive care unit showed that 40 children had a PaO_2 of less than 20 mmHg (2.6 kPa) out of a total 7751 admissions in a 9 year period. Of these, 33 children survived to hospital discharge. There was a U shaped relationship between arterial oxygen tension and mortality.

The results from the survey showed a varied practice. Of the total, 21 respondents $(42\%, 95\% CI : 29.3 - 55.7\%)$ stated that they do not follow specific PaO_2 targets. Of the rest, as equal number $(21, 42\%)$ aimed for targets between 8.1 (61 mmHg) and 10 kPa (76 mmHg). Only 8(16%) aimed for what would be considered a normal range $(10.1 - 13kPa)$. A majority of the units (96%) reported having an alarm target on their oxygen saturation monitor. However, 73% of the respondents worked in units that did not have an oxygen weaning protocol for mechanically ventilated patients. In acute respiratory distress syndrome, cardiac arrest and sepsis, there was a tendency to aim for lower PaO_2 (<10 kPa) as the FiO_2 increased. Following traumatic brain injury and in pulmonary hypertension, there was a propensity to aim for normal PaO_2 (10.1-13 kPa) even as the FiO_2 rose (28-33% when FiO_2 >0.4).

The systematic review identified 11 studies (n=5280) related to hypoxia with combined odds ratio (OR) for death of 3.13 (95%CI: 1.79-5.48, p <0.001) compared to normoxia. Six studies (n=2012) investigated the effect of hyperoxia and suggest no effect on mortality compared to normoxia: OR 1.15 (95%CI: 0.42-3.17, p = 0.77).

Chapter 4

A DropTOI value of 14 was noted to have the least crossover between the mitochondrial disease group and controls. This was empirically set as the cutoff for the 2x2 table. The Fisher exact test statistic was 0.22 and showed that NIRS VOT is unable to differentiate between mitochondrial disease and controls. The NIRS VOT was able to exclude mitochondrial disease in healthy children with a high degree of certainty (positive LR - 5.66, 95%CI:0.84-38, negative LR - 0.61, 95%CI:0.47-0.80).

Chapter 5

The SpO_2 at rest decreased from 99 ± 0.79 to 89 ± 2.54 % from London to Namche (p=0.0009). The SpO_2 after exercise decreased from 93.5 ± 7.16 to 83.3 ± 5.06 % from London to Namche (p=0.008). The heart rate Z-scores at rest changed from -0.44 ± 0.70 to 0.10 ± 0.76 bpm (p=0.30) whilst the heart rate Z-scores after

exercise increased from 1.20 ± 1.30 to 2.07 ± 1.46. Cardiac index (at rest) did not show a statistically significant change with an ascent to moderate altitude. Respiratory rate after exercise increased consistently from sea level (22.2 ± 4.4) to Namche Bazaar (27.6 ± 10.9).

With a multilevel functional regression analysis, the baseline TOI was significantly lower in Namche Bazaar than London (-4.05, 95%CI: -3.23, -4.86, p <0.001). The lowest TOI was also significantly lower in Namche Bazaar (-1.95, 95%CI: -1.13, -2.76, p <0.001). The DropTOI at Namche bazaar compared to sea level was 2.1 points lower (p <0.0001).

Chapter 6

Tissue Oxygen Index varied during the first 5 days of illness. The DropTOI decreased until day 4 (3.02 lower) and showed relative increase on day 5 (0.93 lower) compared to day 1. The net routine control ratio (measured with oroboros analyser) dropped from day 1 to day 5. On the group level analysis (all five patients assessed together), with a false discovery rate (FDR) at <25% and ranked according to their Normalised Enrichment Score (NES), 1039 out of 3655 gene sets showed a decreasing profile with time. The respiratory electron transport chain gene set was ranked 75th of the 1039 gene sets.

Conclusions

The oxygen administration practices in the paediatric intensive care units in the UK vary widely. Of note, admission PaO_2 has a 'U-shaped' relationship to mortality in children admitted to the paediatric intensive care unit. Healthy children, when exposed to hypobaric hypoxic conditions, show a tendency to decrease oxygen consumption from forearm muscle. There was some evidence that critically ill children demonstrate similar signs of early adaptive response by reducing oxygen consumption.

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Chapter 1

Introduction

1.1 Introduction

Critical illness in children may have a long-term impact. Over a three year period (Jan 2010 - December 2012), 57,949 children were admitted across 30 paediatric intensive care units (PICU) in the UK. Although outcomes are improving the number of admissions is increasing. [1] There is a need for research into the most common therapeutic interventions instigated in paediatric intensive care. Supplemental oxygen is one such strategy. Oxygen is usually administered in the setting of hypoxia. Is there enough evidence to support this strategy?

In this thesis, I explored the hypothesis that children adapt to hypoxic condi-

tions during critical illness. In this introduction chapter, evidence on the effect of hypoxia and hyperoxia on outcome in critically ill patients is reviewed. Next, I describe the current theories around hypoxic adaptation in more detail.

I have covered this under the sections:

1. Role of reactive oxygen species
2. How might the body adapt to hypoxic states?

2.1. Animal studies

2.2. Oxygen sensing and Hypoxia Inducible Factor system

3. Studies in Healthy Humans
4. Studies in patients
5. Potential future research strategies

A brief discussion of cardiovascular physiology, oxygen delivery and techniques to measure cardiac output follows. The next section describes the physiology of oxygen consumption and methods of its measurement.

Later I detail the near infrared spectroscopy with a vascular occlusion test (NIRS VOT). The results of this non-invasive technique may be extrapolated to ascertain regional oxygen consumption status. Next, I discuss how oxygen is utilised in mitochondria to generate energy. This is followed by a brief description of the

genetics of mitochondrial diseases. Finally I write a section on my hypothesis for each of the chapters.

1.2 Does Hypoxia Kill?

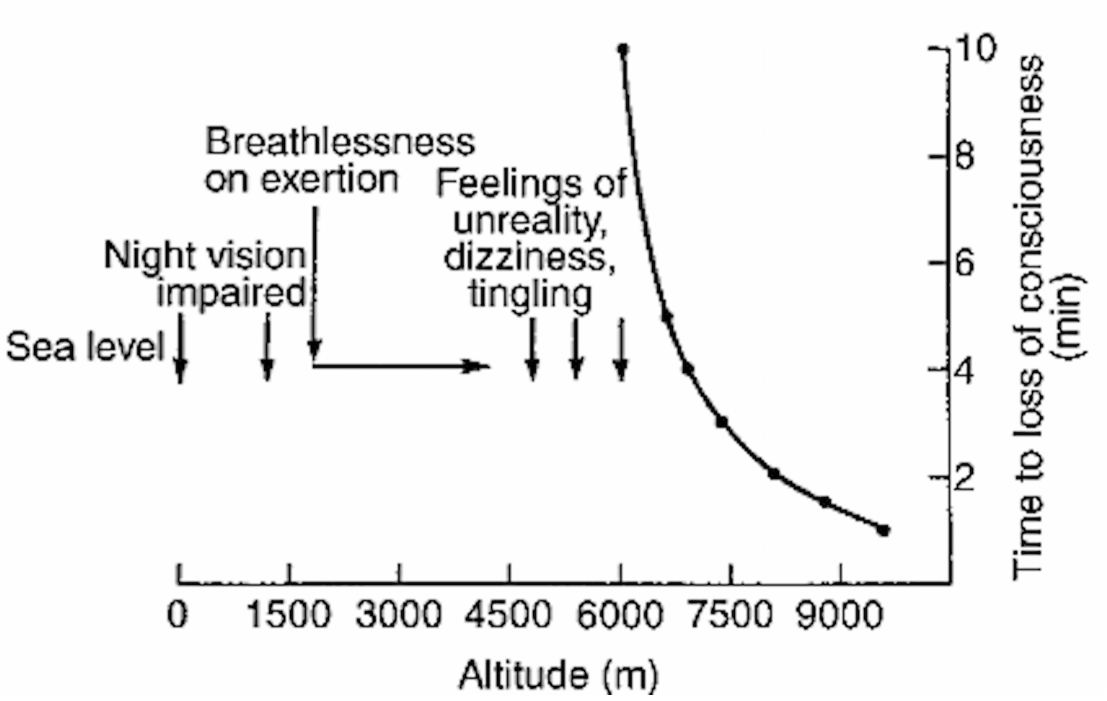
Hypoxia is defined as the condition where the body is deprived of adequate oxygen supply. Hypoxia causes injury due to the cessation of cellular aerobic respiration. Severe hypoxia can lead to cell death and eventually death of the organism. High altitude balloonists were amongst the first to clearly describe the effects of acute hypoxia. These effects were attributed to the low barometric pressure and low partial pressure of oxygen (hypobaric hypoxia). [2] Barcroft classified hypoxia into 3 sub-categories: anoxic type (hypobaric hypoxia), anaemic type (inability to carry oxygen) and stagnant type (hypoxia secondary to circulatory failure). [3] He wrote "*Anoxaemia not only stops the machine, but wrecks the machinery*"

He hypothesised that chronic hypoxia must elicit an acclimatising effect from the human body in the form of an increase in haemoglobin or blood flow. [4] The physiological effects of acute hypoxia, especially when associated with increasing altitude, have been clearly described in the last few decades. Unconsciousness was noted to be a pre-terminal sign of acute severe hypoxia reflecting inadequate

brain oxygenation. [5] Figure 1.1 describes the impact of worsening hypoxia (due to increasing altitude) on the body. It also shows the rapidity of onset of unconsciousness from about 6000 metres above sea level. Although the effects of acute hypoxia are understood, it has been more difficult to delineate the effects of chronic hypoxic exposure.

Figure 1.1: Effect of Hypoxia on the Senses with Increasing Altitude

The x-axis denotes increasing altitude in metres. The vertical arrows point to the approximate altitudes where each of the neurological symptoms manifests. Above 6000 metres, humans lose consciousness. The y-axis denotes the amount of time needed to lose consciousness with increasing altitude. Reproduced from an article by Sharp et al. [5]



The effects of acute and chronic hypoxia on the human body are relevant in the critically ill patient. A recent study in adult intensive care patients demonstrates a direct link between poor oxygenation and eventual outcome. [6] Eastwood et al investigated the Australian - New Zealand adult intensive care unit database. They reviewed PaO_2 in the first 24 hours in 152,680 mechanically ventilated patients. This revealed an increase in the adjusted odds ratio of death with decreasing PaO_2. [7]

But the level at which hypoxia becomes fatal is unclear. Despite the variation among patients admitted to an intensive care unit, an arterial PaO_2 of 2.6 kPa (20 mmHg) was thought to be the threshold below which survival was impossible. [8] Gray et al published a case series of adult acute medicine admissions. They reviewed all arterial blood gas records over a two-year period comprising measurements from 1,700 hospitalised patients. These patients did not receive intensive care. They found 22 patients with one or more PaO_2 values below 2.6 kPa (20 mmHg). Of the 22 cases, 13 (59%, 95%CI: 39-77%) survived to hospital discharge. Six (50%) of these patients were reported to have a good neurological outcome. But the functional outcome of these survivors is not clear. [9]

Grocott et al addressed functional status following exposure to hypoxia. A team of researchers climbed Mount Everest over a 2 month period. They collected arterial blood samples from the femoral artery at an altitude of 8400 metres. The

ability to draw femoral arterial blood, perhaps suggests a good functional status. Incredibly, the results showed that one of the investigators had an arterial PaO_2 of 2.54 kPa (19.1 mmHg). This could be partly explained by acclimatisation. By improving the efficiency of oxygen utilisation humans seem to function even with a lower arterial partial pressure of oxygen. This paper provides further evidence that there are exceptions to the threshold set by Campbell. [10] The evidence to set a lower safe limit of arterial PaO_2 is, therefore, equivocal.

Hypoxia is one end of the spectrum of oxygenation status seen in the critically ill patient. However, a proportion of resuscitated patients in intensive care tend to be hyperoxic. To achieve these values intensivist might resort to aggresive therapeutic startegies. A better definition of the upper limit of PaO_2 might limit the harm caused by these interventions. Therefore, the evidence relating to hyperoxia is addressed below.

1.3 Does Hyperoxia Kill?

Studies in humans report harm due to hyperoxia. Kilgannon et al looked at 4459 patients (>17 years) admitted to 120 US hospitals following cardiopulmonary resuscitation. They observed a linear relationship between in-hospital mortality and post-resuscitation arterial PaO_2 (Odds ratio - OR-1.8, 95%CI: 1.5-2.2). The arterial PaO_2 was included as a continuous measure in this study. Therefore, the

odds ratio refers to any hyperoxia as opposed to per unit change. [11] In a study in children, Ferguson et al reported a non-linear relationship between PICU mortality and hyperoxia (OR-1.25, 95%CI: 1.17-1.37) in those admitted following a cardiac arrest. [12] A recent paediatric study does not support the above findings. [13]
In the last decade, the value of supplemental oxygen in acute coronary syndromes has been questioned. [14] A recent randomised controlled trial in patients with ST-elevation myocardial infarction compared supplemental oxygen (8 L/min) to no oxygen. They found a statistically significant increase in creatine kinase (surrogate for infarct size), troponin I, reinfarction rate and arrythmias in the oxygen treated group. [15] Similarly, adult stroke patients had no difference in 1-year survival between no oxygen and supplemental oxygen therapy. [16] Damiani et al have synthesised these studies in a systematic review. They report increased mortality due to hyperoxia in post-cardiac arrest patients (OR = 1.42, 95%CI: 1.04-1.92), patients following a stroke (OR = 1.23, 95%CI: 1.06-1.43) and patients who suffered traumatic brain injury (OR = 1.41, 95%CI: 1.03-1.94). [17]

1.4 Role of Reactive Oxygen Species

The harm from hypoxia and hyperoxia maybe mainly due to the action of reactive oxygen species (ROS). Reactive oxygen species are chemically reactive molecules

involved in oxidation and reduction reactions. The majority of ROS are generated in the electron transport chain in the mitochondria. The most important of these are the superoxide radical ($O_2^{\cdot -}$), the hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2). [18] Reactive oxygen species are produced by all cells in minute quantities in health. They are needed for redox signaling and homeostasis in cells. Normally, anti-oxidants - such as the superoxide dismutase (SOD) - neutralise these ROS molecules. But in hypoxic and hyperoxic conditions ROS production overwhelms the scavenging system (anti-oxidants). This creates a milieu for cell damage. Reactive oxygen species damage cells by causing oxidative damage to deoxyribonucleic acid (DNA) and cellular proteins and by lipid peroxidation. [19] [20] Oxygen rich environments cause increased production of ROS. [21] Equally, oxygen poor states may also cause ROS production due to reverse electron transport from complex III of the electron transport chain. [22]

1.5 How Might the Body Adapt to Hypoxic States?

The theories behind potential adaptations have been devised from basic physiology research. The Hypoxia Inducible Factor (HIF) pathway is detailed first. I will follow this by discussing animal studies and studies in healthy humans. Finally, I will describe research in patients.

1.5.1 Oxygen Sensing and Hypoxia Inducible Factor System

Semenza, Ratcliff and Kaelin discovered the oxygen sensing system in humans. [23] They noted that cobalt poisoning caused an increase in erythropoietin. It was known that hypoxia stimulates erythropoiesis probably through the activation of an oxygen sensor molecule. Therefore, the suspicion was that cobalt acts on this molecule. This molecule was thought to be limited to cells that produce erythropoietin. Both the Semenza and Ratcliff groups worked on understanding the regulation of erythropoietin gene expression. Eventually, the Semenza group identified the Hypoxia Inducible Factor and realised that it acts on numerous cell types.

Another seemingly unrelated observation was in Von-Hippel Lindau (VHL) disease patients. These patients are prone to develop tumors. The cores of these tumors are hypoxic regions but also stimulate angiogenesis. Some of these patients also demonstrated erythrocytosis. Kaelin hypothesised that the expression of VHL gene caused the downstream effects of angiogenesis and erythrocytosis. He named the proteins formed from this gene expression VHL product (pVHL). In addition, the Kaelin group demonstrated the dysregulation of several hypoxia inducible mRNAs in VHL disease. Therefore, there was a relationship between pVHL and the HIF system. [24]

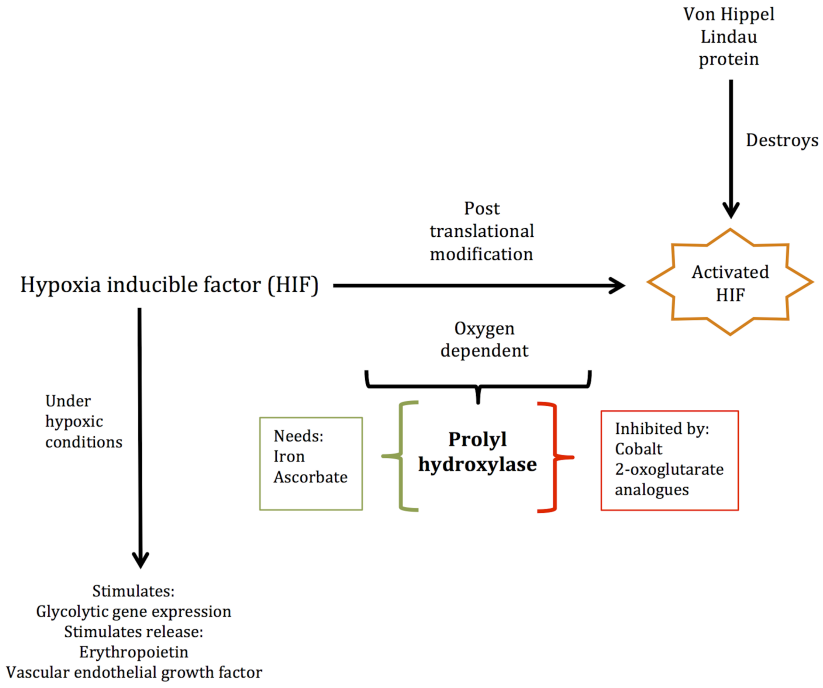
The Ratcliff group reported the link between pVHL and HIFα. They noted that

a physical binding of pVHL and HIFα was necessary for the oxygen dependent instability of HIFα. These findings have led to the current definition of the HIF system. [25]

The HIF is a DNA binding complex. It is made up of α and β heterodimeric proteins. HIF α transduces oxygen sensitive signaling. Before HIF can be deactivated by VHL protein, it undergoes an oxygen dependent post-translational modification. This reaction is catalysed by prolyl hydroxylase enzyme and requires iron, oxygen and ascorbate. Cobalt inhibits this reaction. Therefore, cobalt stabilises HIF and causes an increase in erythropoietin. [23] (Figure 1.2)

Figure 1.2: Oxygen Sensing in Tissues

The Hypoxia Inducible Factor (HIF) is stabilised in the absence of oxygen. This leads to stimulation of glycolytic gene expression and release of erythropoietin and vascular endothelial growth factor. The Von-Hippel-Lindau protein destroys activated HIF. HIF is activated by prolyl hydroxylase in the presence of oxygen.



1.5.2 Animal Studies

Drosophila melanogaster species have been widely employed in the investigation of hypoxic adaptation. They have certain characteristics that make them suitable. These flies have a short life cycle, a short genome and, incredibly, share hypoxia response genes with humans. Furthermore, they have an inherent variable hypoxia tolerance. Interestingly, 70% of known human disease genes are present in their

genome.

Researchers incubated Drosophila flies in 4% oxygen environment. Over generations a hypoxia adapted strain was created. The metabolomics profile was compared between naive flies and hypoxia adapted flies. They observed an increase in glucose, oxaloacetate and alanine in the hypoxia-adapted flies. Furthermore, an increase in ATP production (surrogate indicator of hypoxia-tolerance) with relatively lower oxygen consumption was observed in the hypoxia group. Following this, enzymatic pathways were investigated. They noted an increase in glycolysis. In addition, the flux through complex I increased and there was a suppression of mitochondrial biosynthesis. [26]

The next methodology employed was a reverse gene approach. With this approach, a specific gene expression is altered first. The resulting change in phenotype is analysed. Zhou et al produced hypoxia tolerant flies by exposing them throughout their life cycle to increasingly hypoxic environments over 32 generations. The last generation was able to survive, function and reproduce at 4% oxygen chambers. Some embryos of the hypoxia-tolerant flies were incubated in normoxic conditions for 8 further generations. Incredibly, the embryos of the 9^{th} generation were able to function and reproduce in the 4% oxygen environment. This demonstrated that hypoxia tolerance gene expression was heritable. In addition, gene expression related to carbohydrate and peptide metabolism was down regulated. Phenotypi-

cally, these flies with down regulated gene expression were relatively smaller flies. Furthermore, they noted down-regulation of gene expression of the respiratory electron transport chain, especially complexes I and IV. [27] Mammals also have shown adaptive characteristics to hypoxic stimulus. Investigators have observed increased oxygen carrying capacity, red cell mass and blood volume in animals that are able to cope with hypoxic conditions such as deep sea diving whales. These were in addition to a decrease in basal metabolic rate. These changes are perhaps due to alterations in Hypoxia Inducible Factor (HIF) dependent pathways. [28] I will discuss the HIF system in the next section.

Individual organ systems have also been investigated in mammals. Zhao et al bred rats in a hypoxic environment. This enabled preconditioning of rat neuronal cells. This was followed by a hypoxic-hypoglyceamic insult. They assessed peroxisome proliferator activated $receptor - \gamma$ co-activator $1 - \alpha$ (PGC1 $- \alpha$) and HIF $1 - \alpha$ expression. PGC1 $- \alpha$ is a transcription co-factor. It is found in high concentrations in mitochondria-rich tissues. It is a regulator of cellular energy metabolism. The hypoxia preconditioned cells demonstrated less cell damage, more expression of PGC $1 - \alpha$, HIF 1-α and vascular endothelial growth factor. This protection from hypoxic pre-conditioning was not noted in HIF $1 - \alpha$ deficient cells. [29] In a different experiment, wistar rats were housed in 11% oxygen environment. They showed an increase in haematocrit but no decrease in cardiac mass. Sub-

sarcolemmal mitochondria showed decreased activity of electron transport chain complexes I, II and IV. Significantly, there was a reduction in ROS production and opening of Mitochondrial Permeability Transition Pore (MPTP). The opening of MPTP is associated with ischaemic-reperfusion injury. [30] Therefore, hypoxic preconditioning may protect against ischaemic-reperfusion injury.

Hochachak described the possible mechanisms by which hypoxic adaptation may occur in hypoxia-tolerant animal species (aquatic turtles and fishes). The primary mechanism might be a reduction in ATP turnover in tandem with a decrease in metabolic rate. The balance between ATP demand and supply is perhaps achieved by a decrease in protein turnover and a decrease in urea and glucose biosynthesis. [31] Animals that are not usually exposed to hypoxic environment have also shown adaptive physiology when subjected to low oxygen settings. Beneficial phenotype of hypoxic adaptive response has been noted in rats bred in 10% oxygen environment. These rats demonstrated a 36% increase in red blood cells. This suggests chronic hypoxic adaptation. Stem cells from donor rats from a normoxia group and the hypoxia-adapted group were infused into rats whose bone marrow was ablated with radiation. Both haematopoietic stem cell function and bone marrow engraftment were better when donor rats were from the hypoxia-adapted group. [32]

In summary, evidence from animal studies suggests hypoxic adaptive responses maybe advantageous.

1.5.3 Healthy Human Studies

Healthy humans were exposed to acute hypoxia under strict glucose and lactate control conditions. They demonstrated a reduction in resting energy expenditure. This reduction persisted even after 2.5 hours of cessation of hypoxia. In addition, the glucose requirement was lower in hypoxia group compared to normoxic controls. This suggests that hypoxic adaptive physiology is instigated very early on in the hypoxic phase and remains active well after normoxia is achieved. [33] Another study investigated 7 healthy volunteers housed in a hypoxic environment for 10 days. They observed a significant increase in erythropoietin, haemoglobin and haematocrit levels by day 2 of exposure. These findings are similar to animal studies and probably secondary to the activation of the HIF system. [34]

The human respiratory system responds to hypobaric hypoxic conditions by the hypoxic ventilatory response (seen in sojourners to high altitude). Hypoxia inducible factor causes an increase in erythropoietin and augments erythropoiesis. Hypoxia leads to angiogenesis by stimulating VEGF. Prolonged hypoxia can stimulate changes in the pulmonary vasculature and result in the development of pulmonary arterial hypertension. [35] Although these changes are noticed in different

organ systems the adaptive pathway at the level of the organism is still unclear. Understanding these mechanisms may help us comprehend the changes occurring during critical illness in humans. [36]

1.5.4 Studies in Patients

Cystic fibrosis is a chronic life-limiting condition that involves recurrent chest infections and bronchiectatic changes of lung architecture. This leads to chronic hypoxaemia. In the Boning study, haemoglobin-oxygen affinity between adult patients and healthy controls was compared. The oxygen dissociation curve (ODC) that depicts the relationship between haemoglobin oxygen saturation and partial pressure of oxygen - was shifted to the right. The ODC can shift to the right due to acidosis, hyperthermia and increased 2,3-diphophoglycerate (DPG) concentrations. In fact, Boning observed an increase in 2,3-DPG. These findings suggest a decrease in haemoglobin-oxygen affinity and a relatively easier oxygen release at the tissues. It was postulated that this shift might be secondary to increased erythrocytic glycolysis and red cell turnover that cause a rise in 2,3-DPG. [37] However, a study in a similar chronic hypoxia population shows a different mechanism. [38]

Asthma is a chronic inflammatory condition that might recurrent episodes of hypoxaemia. Hallmarks of the disease are bronchoconstriction and a reduction in

oxygen uptake in the lungs. Of note, there is an increased generation of reactive oxygen and nitrogen species due to the inflammation. Airway cells have fewer mitochondria and seem to be more efficient in an experimental murine asthma model. [38] The oxygen consumption rate from platelets was measured in a study comparing 12 asthma patients (not severe) and 13 healthy controls. The mitochondrial DNA copy number per platelet was not different between the groups. The basal respiration, proton leak and ATP linked respiration remained equal. The oxygen consumption rate in the asthma patients was stable and independent of glycolysis. Increased aconitase levels were observed in asthma patients. This suggests increased tricarboxylic acid cycle (TCA) activity. The substrates of TCA cycle fuel the oxidative phosphorylation in mitochondria: potentially this can augment oxygen utilisation in the mitochondria. [39] In patients with severe obstructive sleep apnoea and nocturnal hypoxia, VEGF levels were higher compared to healthy controls. Furthermore, there was a linear correlation between oxygen saturation and level of VEGF. [40] The elevated levels of VEGF might be secondary to activation of the HIF system. If VEGF was stimulated then, other effects of the HIF system might also be activated such as erythropoietin release. Hence, VEGF levels may potentially be a marker of hypoxic adaptation. These studies concur in part with evidence from animal research. [26] Unfortunately, the metabolomic changes in chronic hypoxia patients have not been well investigated

and need further research.

1.6 Potential Future Research Strategies

Several avenues are currently being explored to delineate hypoxic adaptation pathways. I will mention a few promising areas here.

1. Nitric oxide (NO) is central molecule in several metabolic pathways including hypoxic adaptation. Nitric oxide causes vasodilation under conditions of tissue hypoxia. The aim is to increase oxygen delivery. Studies on Sherpas concur with this theory. The level of circulating NO and forearm blood flow in Sherpas was noted to higher compared to low landers. [41] The complimentary effect to vasodilation is an inhibition of cellular metabolism. NO inhibits complex I and competes with oxygen at the cytochrome c oxidase site in the electron transport chain. These effects have led researchers to augment NO synthesis and investigate its impact on oxygen consumption. Different research groups have reported contradictory findings. [42] This suggests that the pathways that interact with NO synthesis and function are still unclear. Research in this area is being actively pursued. [43]

2. Micro Ribonucleic acids (miRNA) are present in all eukaryotes. They are non-protein coding and regulate gene expression. Recent discoveries propose an important role for miRNAs in hypoxic adaptation. Whilst HIF 1- alpha causes metabolic adaptations by transcriptional regulation, miRNA work at the post-

transcriptional level. The two systems may work in tandem to accomplish hypoxic adaptation. The miRNA may be particularly helpful in transient and local hypoxic challenges. [44] miR-210 is a hypoxia-regulated miRNA that is expressed in all cell types. [45] It targets specific proteins needed for the functioning of the mitochondrial electron transport chain and the TCA cycle. Under hypoxic conditions, miR-210 causes repression of protein function. This results in relatively more ATP formation for the oxygen available through augmented glycolysis. [46] Therefore future research in understanding the exact pathways by which miRNAs act will help direct therapeutic strategies to perhaps augment their function.

3. One therapeutic strategy that might help hypoxic patients is the use of dexamethasone. Prophylactic use of dexamethasone in mountaineers has ben observed to reduce the risk of high altitude pulmonary edema. The mechanism of action might be a direct augmentation of ventilatory acclimatisation to hypoxia. The proposed mechanisms that might cause this are increased surfactant production and an increase in the sodium transporters in the alveoli. [47] In addition, dexamethasone may up regulate oxidative phosphorylation gene expression. [48] Finally, dexamethasone probably acts on the HIF system and decreases the production of erythropoietin. In turn, there is a reduction in haematocrit and viscousity aiding in reduction of pulmonary vascular resistance. [49] In chronic hypoxic patients, early utilisation of dexamethasone might reduce the risk of developing right heart

failure secondary to pulmonary hypertension.

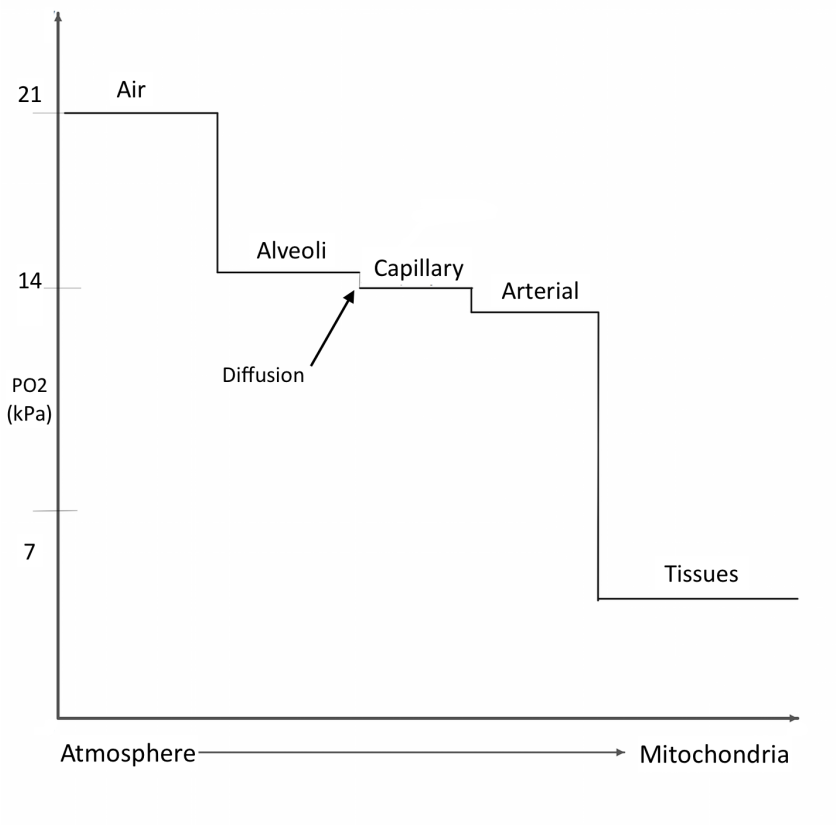
In subsequent sections, I will detail the oxygen cascade, the Doppler ultrasound cardiac output measurement technique, the principles of near infra-red spectroscopy and basic mitochondrial physiology.

1.7 The Oxygen Cascade

The cardiovascular and respiratory systems work in concert to deliver oxygen to the tissues and to wash out metabolites from the tissues. The delivery and consumption of oxygen by the body are in series. The oxygen pathway starts with the inspired air (21% at sea level). With inspiration, oxygen travels to alveoli. From alveoli, a step-wise cascade transports it to the tissues. Figure 1.2 illustrates this cascade. [50] The main determinants of oxygen delivery to tissues are the Alveolar-arterial oxygen (A-a) gradient, the cardiac index, haemoglobin concentration and arterial oxygen saturation. We will now consider the measurement of oxygen delivery by incorporating these parameters into a single model.

Figure 1.3: The Oxygen Cascade

The x-axis denotes the steps an oxygen molecule takes leading from the atmosphere to the mitochondria. The y-axis depicts the gradual drop in the partial pressure of oxygen at the various sites. Amended from Raman et al. [50]



1.7.1 Oxygen Delivery

Oxygen delivery (DO_2) can be calculated by measuring the various components necessary for transfer to the tissues. The formula incorporates haemoglobin (Hb), haemoglobin oxygen saturation (HbO_2), cardiac output (CO) and the partial pressure of arterial oxygen in blood (PaO_2). The formula is as follows:

$$DO_2 = CO \times CaO_2 \tag{1.1}$$

where

DO_2 is measured in mls of O_2/min

CO denotes cardiac output measured in L/min and

CaO_2 denotes oxygen content of arterial blood.

The oxygen content can be calculated by the formula:

$$CaO_2 = (1.36 \times Hb \times SaO_2) + (0.0031 \times PaO_2) \tag{1.2}$$

where

Arterial oxygen content is measured in ml of O_2/100ml of blood,

Hb denotes haemoglobin measured in grams/decilitre,

SaO_2 denotes saturations from arterial blood gas measured as percentage,

PaO_2 denotes arterial oxygen tension measured in mm Hg

the constant 1.36 is the amount of oxygen bound to each gram of hemoglobin

the constant 0.0031 is the amount of oxygen dissolved in plasma

A major determinant of oxygen delivery is the cardiac output. I will discuss the

measurement of cardiac output in further detail now.

1.7.2 Cardiac Output

Cardiac output (CO) is the amount of blood pumped out of the heart in a minute. This is a product of the heart rate per minute and the stroke volume (blood volume per heart beat). Cardiac output is usually expressed as cardiac index (CI) (CO per unit body surface area). Cardiac index is crucial to ensure oxygenation of tissues. The normal range of CI in humans is between 3.5-6 L/min/m^2. Clinicians consider cardiac output as a marker of the global oxygen demand-supply relationship. [51] In a study in septic adult patients, low cardiac output (set at <4.5 L/min/m^2) was demonstrated to have worse outcome (74% vs. 40%, p <0.05). [52] In the landmark study from Rivers, patients' central venous oxygen saturation was optimised by measuring and supporting their cardiac output. [53] The association between measuring/ supporting CO and influencing outcome is unclear. A patient may have a cardiac output in the normal range, but still be in a negative oxygen demand-supply relationship. LeDoux et al proved, in their septic model, that augmenting perfusion pressure does not improve tissue perfusion or outcome. [54] This finding may be due to the varying relationship of oxygen delivery (DO_2) and consumption (VO_2). I will explore this relationship later in chapter 6.

Methods of Measurement of Cardiac Output

The clinical signs used to assess cardiovascular function are useful, but inaccurate at times. A recent study substantiates this view. [55] Adolf Fick was the first to devise a solution to measure cardiac output. He described the measurement of cardiac output by using the universal principle of law of mass diffusion. [56] His technique has been advanced further in the last century. In the present day, some of the following techniques are used to measure cardiac output:

A. Thermodilution technique

B. Lithium dilution techniques

C. Arterial pressure wave / contour analysis techniques

D. Technique using ultrasound and Doppler

I have used the ultrasound doppler techinque in my thesis. This is discussed in more detail here.

Ultrasound Doppler Cardiac Output Measurement

The Doppler principle in conjunction with ultrasound has enabled the non-invasive measurement of cardiac output. Two methods use this principle: 2 D Echocar-

diography and Ultrasound Cardiac Output Monitor (USCOM). Echocardiography provides a more in-depth analysis of the cardiovascular status. However, it is impractical in an intensive care unit. This is partly due to the prolonged training needed to become proficient in echocardiography. In addition, it is time-consuming and resource intensive. Most intensivists prefer a non-invasive bedside tool. Consequently, USCOM becomes appealing.

USCOM has a major inherent assumption. It assumes that the aortic valve acts as a fixed circular outlet. There are some reservations to this assumption, as explored later. If we were to consider a circular tube - akin to the aortic outflow - the formula to measure the mass is:

$$Mass = Volume \times Density \tag{1.3}$$

We can assume the density of blood coming out of the heart is constant and hence can be treated as '1'. Therefore, the formula can effectively be considered to be:

$$Mass = Volume \tag{1.4}$$

At the aortic valve, this volume will be a measurement of the stroke volume. The next stage is to measure the volume.

According to fluid dynamics,

$$Volume = A \times v \times t \tag{1.5}$$

where

A denotes Area,

v denotes Velocity and

t denotes Time.

As the cardiac output is measured at the aortic valve, the area of the tube considered in our model is known. This is the area of aortic valve annulus. By the use of echocardiography measurements and body surface area, nomograms of the aortic valve annulus have been created for both adults and children of all ages. The main reservation is the lack of standards for measurement of the aortic valve diameter (distance between outer edges or inner edges) and also whether the measurement should be made during systole or diastole (the annulus width is not constant throughout the cardiac cycle). [57] [58] [59]

The measurement of Velocity Time Integral (VTI) is feasible by directing the ultrasound wave at the aortic valve. This is possible because we can measure the flow and the time it takes for the Doppler wave to hit the blood outflow from the heart and return to the probe. Some authors have suggested using nomograms of the aortic flow velocity integral instead of aortic valve area for more accurate and

individualised measurement of the cardiac output. However, these need further exploration. [60] Despite its limitations, USCOM has nevertheless found clinical utility due to the simplicity and non-invasive nature of this method.

Researchers have proven the validity of USCOM in several settings. An animal study measured cardiac output by USCOM and ultrasonic flow probe placed directly over the ascending aorta in 6 anaesthetised dogs. The mean bias (Bland-Altman method) was $-0.01\ L/min$ and the limits of agreement were -0.34 to $0.31\ L/min$. In 5 of the 6 dogs, Lin's coefficient of concordance was >0.9. [61] A study on children (<12 years old) who underwent elective surgery similarly showed a high concordance correlation coefficient of 0.87 for inter-observer variability. It also showed a small intra-observer variability. [62] A validation study in adults comparing USCOM to the thermodilution technique using a PAC showed a reasonable correlation at 0.8. [63]

Although there are reports of the validity of USCOM in the theatre setting it will be ideal to show this in the emergency department or the intensive care unit. A study in children presenting to an emergency department has addressed one of these issues. This study included the examination of 41 children. The procedure was successful in 94.7 % of the children and yielded acceptable records in 75.9 % of these. However, the kappa value for clinical correlation of USCOM measurements was low between two raters. [64] Most operators believe USCOM to give reason-

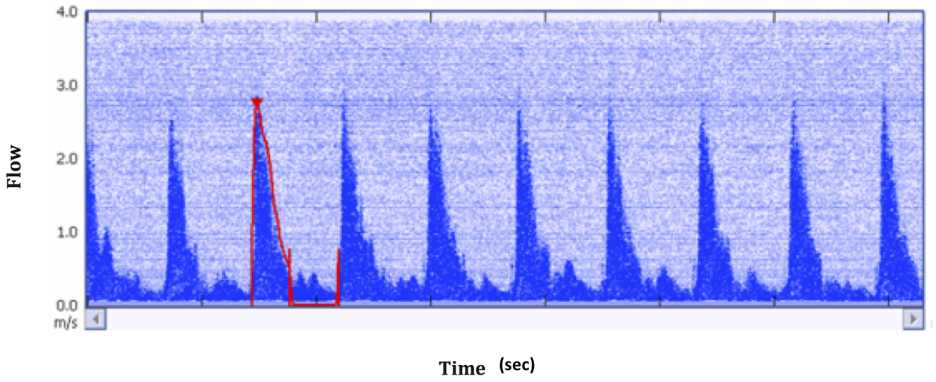
ably reliable values in children and adults despite the issues surrounding intra and inter-observer variability.

A study in premature neonates showed poor correlation and reliability. [65] A meta-analysis including 10 studies in children and adults in a variety of clinical settings reported 42.7 % (95 % CI: 38.5-46.9 %) error. This is similar to other minimally invasive techniques of cardiac output measurement. Of note the mean weighted bias between USCOM and thermodilution techniques was low at -0.39L/min ($95\%CI$: -0.25 to -0.53 L/min). [66] Normal ranges of USCOM measurements are available both for children and adults. [67] [68]

A recent study, set in a tertiary paediatric intensive care unit, supports the use of USCOM in children in shock. The investigators demonstrated the ability to monitor progress and titrate vasopressor and inotrope therapy using the cardiac output and systemic vascular resistance index (SVRI) measurements from USCOM. [69] Figure 1.1 shows a representative recording from USCOM. [70]

Figure 1.4: Example Ultrasound Cardiac Output Monitor (USCOM) Waveform

The x-axis denotes time and the y-axis depicts the flow noted by the probe. Each triangular wave represents one heartbeat. The cardiac output is measured by calculating the area within the triangular waveform. Reproduced from USCOM product guide. [70]



1.7.3 Oxygen Consumption

Oxygen consumption depends on cellular respiration. The amount of oxygen utilised by the cells to generate adenosine triphosphate (ATP) - by oxidative phosphorylation system in the mitochondria - is a major determinant of consumption. Non-mitochondrial oxygen consumption accounts for a small proportion of the total oxygen consumed. Therefore, the oxygen consumption might potentially act as a marker of mitochondrial function and the functional status of cells.

The oxygen extraction rate (OER) is the ratio of oxygen consumption to oxygen delivery. The OER is can be employed to analyse oxygenation of tissues.

$$OER = \frac{VO_2}{DO_2} \tag{1.6}$$

where

OER denotes oxygen extraction rate,

VO_2 denotes oxygen consumption and

DO_2 denotes oxygen delivery.

1.8 Surrogate Measures of $VO_2 - DO_2$ Balance

Some surrogate measures such as lactate anad mixed venous saturations have been employed to analyse the $VO_2 - DO_2$ balance.

A. Lactate

Normal arterial serum lactate ranges between 0 and 2 mmol/L. The production of lactate occurs when the tissues have an insufficient supply of oxygen. Glycolysis for energy production is not optimal in these low perfusion / low oxygen states. The body is forced to produce ATP (albeit inefficiently) by anaerobic pathways. The end product of this anaerobic reaction is lactate. Hence, an increase in lactate can be a marker of low perfusion or low oxygen states. However, the interpretation of high levels must be made with caution. Not all scenarios of high lactate are low perfusion states. For example, in the setting of acute severe asthma one of the common therapeutic interventions is to administer nebulised or intravenous salbutamol. Despite the peripheral oxygen saturation being in the high 90s, ma-

jority of the patients display high lactate levels. Salbutamol causes the generation of lactate by an as yet unexplained mechanism. [71]

B. Mixed venous oxygen saturation

The 'true' mixed venous oxygen saturation is measured from a pulmonary artery catheter placed in the main pulmonary arterial trunk. Blood returning to the right side of the heart contains the residual oxygen after utilisation by the tissues. This makes the $MxVenO_2$ an indirect measure of whole body tissue oxygen consumption. Acute changes in basal metabolic rate and the cardiovascular support needed can be determined from $MxVenO_2$. As noted earlier the pulmonary artery catheter has fallen out of favour.

The next best available site of measure for the venous oxygen content is from the superior vena cava (SVC). One can get as close to the 'true' value as safely possible by positioning the central line tip at the SVC and the right atrial junction. A reasonable correlation exists between the mixed venous oxygen saturation measured from SVC and pulmonary artery. [72] This technique is widely used in the adult emergency medicine and intensive care setting. However, placement of central venous lines, especially in the internal jugular vein, is uncommon as part of routine management of a critically ill child on a paediatric intensive care unit. This measure loses its significance when the blood is sampled from a femoral venous line.

Both lactate and mixed venous saturations are from the vascular compartment. Each tissue receives blood in proportion to its metabolic rate. For example, brain receives three times as much oxygen delivery as its consumption. [73] Therefore, using surrogate markers of oxygen extraction ratio from the vascular compartment may not be an appropriate method to measure oxygen consumption. The 'ideal' way to analyse the $VO_2 - DO_2$ balance would be to delineate this at a regional organ system level and at the whole body level. Unfortunately, there are some practical issues in the search for such a technique. Foremost, it has to be of use in a clinical setting. Further, the technique should be able to analyse tissues from different compartments and compare the results. Unfortunately, these complexities make it impossible for any one technique to be the answer. Therefore, selected a lab-based method for oxygen consumption measurement from peripheral blood mononuclear cells.

1.9 Measurement of Oxygen Consumption

All reliable methods to measure oxygen consumption are invasive. One non-invasive technique that might be a surrogate for regional oxygen consumption measurement is near infrared spectroscopy.

1.9.1 Invasive Methods of Measurement of Oxygen Consumption

Direct laboratory measurements:

Leland Clark was the first to measure oxygen consumption (1953) in a solution using an oxygen electrode after suspending the tissue in a closed chamber with an oxygen restricted medium. He created a platinum electrode coated with silver chloride. A Teflon membrane separated it from the reacting solution. Reduction occurs at the tip of the electrode when oxygen molecules diffuse through the membrane. The flow of electrons due to this chemical reaction generates an electrical current. A potential difference measures this current. He was then able to plot this potential difference as a curve. [74] [75] This technique has evolved in the last half-century.

Two commercially available analysers perform measurement of oxygen consumption from different types of tissues. These are the Seahorse analyser and the Oxygraph-2K high resolution respirometry analyser.

A. *Seahorse analyser*:

The Seahorse analyser works by creating a micro-environment around sensor probes in the micro-chamber. It analyses the medium surrounding a single layer of cells. It measures the rate of oxygen consumption (OCR) and the rate of acid efflux

(ECAR). The measurement of proton leak and the concentration of dissolved oxygen produces these results. The OCR is a marker of the mitochondrial respiration. The ECAR is a marker of the glycolytic metabolism. [76] [77]

B. *Oxygraph-2k analyser*:

The Oxygraph-2K works on a similar principle as the Sea horse analyser. It analyses two samples at one time (two chambers). Researchers have reported analysis of nearly all human tissues with this analyser. The most common tissues in use are muscle and skin samples. Peripheral polymorphonuclear cells (lymphocytes) and platelets have also been used for analysis. [78] The Oxygraph-2K analyser will be discussed in further detail in the methods chapter (section2.3) and under the 'Variation in oxygen consumption during paediatric critical illness' chapter (Chapter 6).

Oxygen consumption in the mitochondria is proportional to intra-mitochondrial ATP levels. ATP levels are linked to oxidative phosphorylation (OXPHOS) activity. [79] An association between OXPHOS activity and gene expression has been noted in patients with mitochondrial myopathies. [80] Therefore, analysing the expression of OXPHOS genes is a way of assessing mitochondrial function. I have utilised this method to analyse children admitted to the intensive care unit. This will be discussed in further detail in chapter 6.

As already noted, mitochondrial function can be estimated by measuring the oxygen consumption of a tissue. I will now investigate the ways to measure oxygen consumption in different tissues.

1. Blood - The measurement of oxygen extraction from the blood is complex. Firstly, the different components of blood extract oxygen at different rates. Secondly, the mitochondrial content is variable. The idea that one can measure the oxygen extraction from whole blood with an oxygen analyser may be simplistic and probably partly erroneous. Researchers have grappled with this issue for more than a decade. The search was for a biomarker that can quantify the mitochondrial content. Following testing of numerous markers, cardiolipin content followed by citrate synthase activity has shown the greatest promise. [81]

Citrate synthase activity is used widely for two main reasons: (a) It gives a reasonably accurate measure of the mitochondrial content and (b) there is no published report to date of an isolated citrate synthase deficiency being compatible with life. Therefore, by separating whole blood into its components and measuring the citrate synthase activity (hence the mitochondrial content) we can quantify the oxygen extraction more accurately.

2. Muscle and other tissues - By utilising the same principle as used for analysis of blood one can analyse any tissue sample. The only additional step needed is

the permeabilisation of the tissue. [82] However, different tissues of the body have different OXPHOS activity. [83]

1.9.2 Non-invasive Surrogate Measure of Oxygen Consumption

The invasive technique detailed above are the 'gold standard' for analysis of oxygen consumption blood, muscle and other tissues. In the paediatric intensive care non-invasive techniques are preferred. Nuclear magnetic resonance spectroscopy is a popular method used in large research units. Another variant is the functional MRI (fMRI) with blood oxygen level dependent effect (BOLD). This technique has been widely used in neuroscience. [84] Scientists have also measured oxygen consumption in the brain in animal models by using fluorescence microscopy. [85] The only available technique for use as a bedside non-invasive surrogate measure in the paediatric intensive care setting is Near Infra Red Spectroscopy (NIRS).

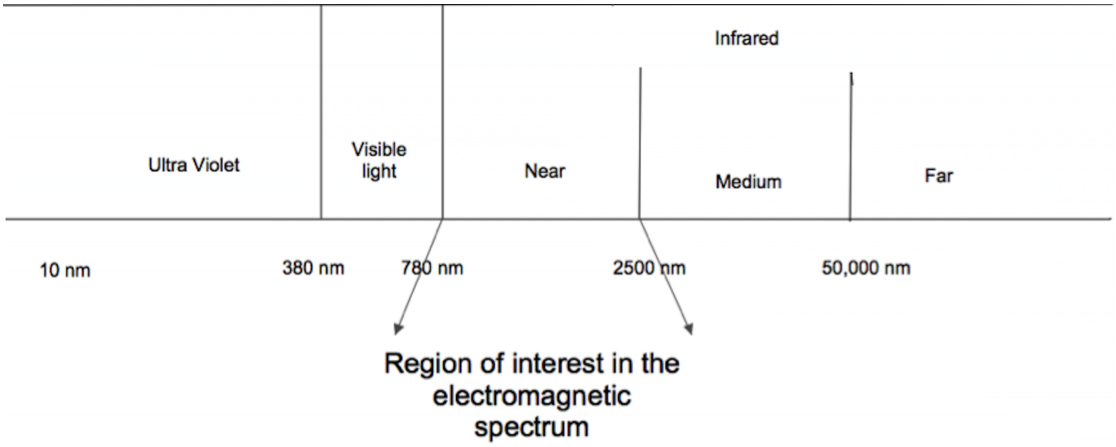
1.10 Near Infrared Spectroscopy (NIRS)

1.10.1 The Physics of Near Infrared Spectroscopy

Two key parameters of human tissues make NIRS feasible. Firstly, human tissue is translucent to light. Secondly, these tissues contain proteins that are variable absorbers of light depending on their oxygenation state. [86]. Near infrared (NIR) light has a wavelength between 780 and 2500 nanometres. Most commercially available NIRS machines use NIR light in the region of 700 to 1000 nanometres. In this range the attenuation of light, whilst passing through tissue, is low. This creates an ideal region of the electromagnetic spectrum in which to investigate tissues.

Figure 1.5: Near Infrared Region of the Electromagnetic Spectrum

The electromagnetic radiations are separated into different components depending on their wavelength. This ranges from ultraviolet to far infrared regions.



The Beer-Lambert law describes the effect of a dissolved compound on the light passing through a solution. Accordingly, the attenuation of incident light is proportional to the wavelength and the concentration of the compound dissolved in solution (where the solution itself does not have any effect on the light).

$$A = \epsilon \times C \times d \tag{1.7}$$

where

A is the attenuation of incident light,

ϵ is the extinction coefficient,

C is the concentration of the absorbing compound in solution and

d is the distance between the point of entry of the light and the point where it leaves the solution.

If there were to be more than one absorbing compound in the solution the attenuation will then be proportional to the sum of the individual effects of each of the compounds on the incident light. In the human tissue there are certain compounds that attenuate light, but have a fixed effect with regards to time or oxygenation state (melanin, water and lipids). These compounds are 'fixed absorbers' and can be safely ignored for the final analysis of tissue oxygenation. The importance of these compounds, especially water, is that they absorb more of the incident light at certain wavelengths. Therefore, it will be prudent to select the wavelengths for our investigation in a region not concomitant with this range. The above scenario works well in a non-biological medium. However, human tissues create inherent complexities. [86]

In human tissues there is absorption, scatter, and reflection of light. The reflected and the scattered component of the incident light is finally received by the optode. The standard Beer-Lambert law is inapplicable in this setting. The modified Beer-Lambert law explains this scenario. It incorporates two differences in the tissue. The first is to account for the loss of incident light due to scattering. The second

is to incorporate the extra distance travelled by the incident light due to scatter within the tissue.

$$A = (\epsilon \times C \times d \times B) + G \tag{1.8}$$

where

A denotes attenuation of the incident light,

ϵ is the extinction coefficient,

C denotes the concentration of the absorber,

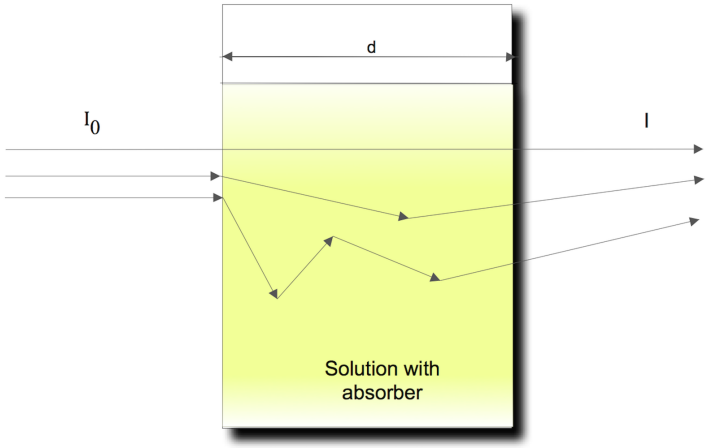
d denotes the geometrical pathlength,

B denotes the pathlength correction factor and

G is the additive term for scattering losses

Figure 1.6: The Modified Beer-Lambert Law

The figure depicts a container with a solution. The solution has an absorber dissolved in it. The incident light with an intensity of I_0 is absorbed, scattered and reflected as it passes through the solution and exits with an intensity I. d stands for the absolute path length.



The scattering losses (G) are not measurable. To overcome this limitation the formula has to be slightly modified. If we were to measure the change in the concentration of the absorber instead of the actual concentration, then the absolute value of G is no longer needed. Let us say we measure the attenuation for absorber C1 (A1) and then add another absorber C2 with a different attenuation (A2). The equation 1.8 changes to:

$$\Delta\ A = \Delta C \times \epsilon \times d \times B \tag{1.9}$$

where

Δ A denotes the differential attenuation

Δ C denotes change in the concentration of the absorber

It is evident that 'd' and 'B' are still necessary to solve the equation. It is easy to measure 'd'. It is the absolute distance between the point of entry of light into the medium and the point of exit. But 'B' (the differential pathlength factor) depends on the amount of scatter which in turn is specific to each tissue. One can use different techniques to measure 'B'. This can be by measuring the change in time of flight of light, change in frequency or measuring the tissue absorption with a known quantity of water. [87] [88] [89]

Several studies furnish values for 'B' for cerebral and forearm measurements in adults. But the values for neonates and children are limited. We have extrapolated from adult and neonatal studies. [88] [90] [91] [92] We will explore the absorbers that NIRO-NX measures.

1.10.2 History of Near Infrared Spectroscopy

The first report utilising NIRS to investigate tissue oxygenation was by Jobsis. [93] The studies exploring cerebral oxygenation in neonates and patients undergoing surgery yielded promising results. [94] [95] These were followed by investigation

into skeletal muscle oxygenation. One of the first studies observed change in forearm muscle oxygenation with venous occlusion (occlusion of venous drainage whilst arterial flow is preserved) for 8 minutes. Near infrared spectroscopy was able to measure dynamic changes in tissue oxygenation. [96]

Blasi et al suggest a measurement of forearm blood flow with venous occlusion. [97] The next step in the advance of NIRS in clinical practice was to use it in conjunction with a vascular occlusion test. [98] There seems to be a correlation between organ failure scores and rate of deoxygenation after vascular occlusion in septic adults. [99] This also extends to a correlation with outcome. Septic patients with lower rates of increase in tissue oxygen after arterial occlusion (slope) had a higher mortality. The baseline slope was 3.3 ± 1.4 %/sec compared to nonsurvivors' slope of 2.4 ± 1.5 %/sec. This difference was persistent until 48 hours after admission. [100] These and numerous other studies have led clinicians to believe that NIRS can be a measure of cardiovascular status. [101] NIRS has also been widely used in children with critical illness secondary to infective aetiology, trauma, and post cardiac surgery. [102] The physics behind NIRS technique will be reviewed now.

1.10.3 Parameters Measured by Near Infrared Spectroscopy

The NIRO-NX machine (Hamamatsu, figure 1.8) was employed for my NIRS measurements. [103] The chromophores (absorbers in a biological tissue) in the human body can be differentiated into static and non-static absorbers. The 'static absorbers' (such as bilirubin) can be ignored as their absorption does not change with occlusion of blood flow. From the non-static measures NIRO-NX gives four outputs: Oxygenated Haemoglobin (O2Hb), Deoxygenated Haemoglobin (HHb), Tissue Oxygen Index (TOI), and net Total Haemoglobin Index (nTHI). The NIRO measures oxygenated and deoxygenated haemoglobin and calculates the others. NIRO is able to give a continuous measure of TOI by utilising the varied absorption spectra of haemoglobin depending on the oxygenation state.

Figure 1.7: The NIRO-NX 200 Machine

Reproduced from the product leaflet from the Hamamatsu inc. [103]

The total haemoglobin index (THI) is calculated by the formula:

$$THI = O2Hb + HHb \tag{1.10}$$

From the O2Hb and THI the NIRO calculated the tissue oxygen index (TOI) by the formula:

$$TOI = \frac{O2Hb}{THI} \tag{1.11}$$

Another parameter of note is the 3:1 representation of venous and arterial compartments during NIRS TOI measurement. A study of cerebral oxygenation validated this ratio. [104] In keeping with this, one can consider TOI as a closer measure of mixed venous saturations.

1.10.4 Limitations of NIRS

NIRS technology has been in use in clinical practice for more than 3 decades, but lacks robust trial data to support its routine use. In addition, there are some technical limitations as outlined below.

1. Adipose tissue under the skin can affect the NIRS measurements from skeletal muscle. [105]

2. Pathlength of light changes during and after arterial occlusion. This change will be nearly consistent across all subjects. This will have a negligible effect as the change is possibly less than 8 %. [106]

3. I used a fixed differential pathlength factor for all ages. This is an assumption based on literature as no report exists for the exact values for various ages. This assumption only affects the Δoxyhaemoglobin (ΔO2Hb) and Δdeoxyhaemoglobin (ΔHHb) values. The TOI and THI use the spatially resolved spectroscopy (SRS) principle. Spatially resolved spectroscopy measures the change in absorption at several wavelengths and at several pathlengths (emitter-receiver distances). The

TOI is then calculated from the relative absorption coefficients of oxygenated and deoxygenated haemoglobin. By using several pathlengths SRS is able to account for some of the constraints of the modified Beer-Lambert law.

4. Haemoglobin (Hb) and Myoglobin (Mb) have similar absorption spectra. Delineating each of their contributions to TOI values is not possible. As desaturation of Mb is minimal at 3 minutes of vascular occlusion, one can assume that the TOI values will represent changes in oxygenation of Hb. [107]

The measurement of oxygen consumption acts as a surrogate marker of mitochondrial function. Having considered the parameters of the oxygen cascade and the cardiovascular physiology in health (section 1.4), I will discuss how this knowledge can be translated to the critically ill patient.

1.10.5 Oxygen Delivery - Oxygen Consumption Relationship During Critical Illness

Shoemacker investigated the cardiovascular physiology during critical illness. He studied the changes in haemodynamic variables in 180 patients with a mixture of aetiologies (haemorrhagic shock, sepsis and trauma) leading to critical illness. Oxygen consumption was measured as the product of arterio-venous oxygen content difference and cardiac index. Oxygen delivery was measured as the product

of cardiac index and arterial oxygen content. He described a changing pattern of cardiovascular response with time during illness by investigating the link between oxygen consumption and oxygen delivery. [108] This led to a comparison study of survivors and non-survivors with critical illness. Outcomes of post-operative patients with circulatory shock were compared by analysing 26 variables in 67 survivors and 31 non-survivors. The period of illness was divided into early, middle and late stages. A clear distinction in the cardio-respiratory pattern between survivors and non-survivors was demonstrated. Survivors had a higher cardiac output, left ventricular stroke work, oxygen availability and consumption. [109]
In a further study, investigating 113 critically ill postoperative patients, Shoemacker et al used the predictive model from the previous study. They reported that efficiency of tissue oxygen extraction (ETOE) correctly predicted outcome in 91% of cases. The efficiency of tissue oxygen extraction was calculated by dividing arterial-venous oxygen content and red cell mass. This had a high predictive coefficient through all the stages of critical illness. [110] Hence, oxygen extraction seems to be an important parameter during the evolution of critical illness. Clinical states such as hyperthermia and certain toxins may increase the oxygen extraction in the tissues. Aggressive normalisation of haemodynamic targets may be helpful in only a subset of patients. [111] Oxygen consumption is a surrogate for OXPHOS activity. Further, OXPHOS activity is linked to OXPHOS gene expression. There-

fore, oxygen consumption is modulated by OXPHOS gene expression (described earlier under section 1.9.1.B). An increased oxygen extraction in the critically ill patient might suggest an efficient mitochondrial function. I have investigated the change in drop TOI (as a marker of oxygen consumption) during critical illness to investigate this hypothesis (chapter 6). I will now discuss the OXPHOS system in more detail.

1.11 Mitochondria: The Site of Oxygen Utilisation

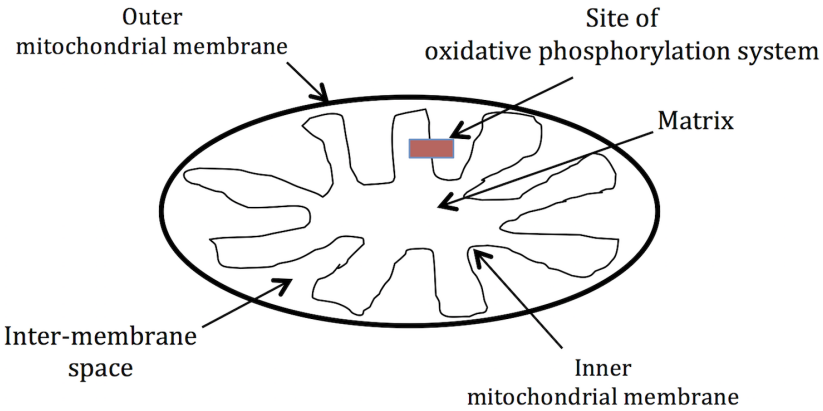
Mitochondria are structures present in almost every cell of the body (except mature red blood cells). Their main function is the production of energy-rich ATP compounds and utilisation of breakdown products from carbohydrate, fat and protein metabolism. In addition, mitochondria have roles in cell apoptosis, in balancing the cellular reactive oxygen species and anti-oxidant status. [112]

The number of mitochondria within each cell varies considerably between tissues. The tissues that have a high energy demand such as the heart, brain and muscles have a proportionally higher number. [83] The structure and morphology of mitochondria are also variable. After the advent of the electron microscope this aspect of the mitochondrion was described. It is formed of an outer membrane,

an intermembrane space, an inner membrane, and an internal matrix. The inner membrane forms baffle-shaped structures called cristae. [113] Recent evidence from yeast and human cells suggests that mitochondria are dynamic structures. They are in a continuous dance of fission and fusion, forming a reticulum. [114] [115] [116] This dynamic nature of the mitochondrion is also implicated in the pathophysiology of diseases. [117] Fission and fusion events within the mitochondrion help to balance the electrochemical milieu and create the right environment for the efficient functioning of the OXPHOS system. The whole structure of the mitochondrion is also dynamic. Mitochondria elongate in times of increased oxidative stress or nutritive deficiency. [118] [119] Conversely, they fragement and uncouple the phosphorylation and electron transport chain during nutritive excess. [120]

Figure 1.8: The Structure of Mitochondrion

The mitochondrion has two membranes, a inter-membrane space, and a matrix inclosed by the inner membrane. The oxidative phosphorylation system is situated on the inner membrane.



1.11.1 The Oxidative Phosphorylation System

The 'Chemiosmotic theory' hypothesised (1966) that the electron transport chain is coupled to phosphorylation. [121] [122] [123] The OXPHOS system is composed of two parts: the electron transport chain and the ATP synthase. [124] It is situated on the inner mitochondrial membrane. It is made up of five multiple subunit enzyme complexes (complex I to IV and the ATP synthase) that participate in the production of energy. The complexes I, II, III, IV and V (ATP synthase) each have 44, 4, 11, 14 and 16 subunits respectively. [125] [126]

Complex I

This (NADH:Ubiquinone oxidoreductase) is the largest of the 5 enzyme complexes. It was first isolated in 1962 from bovine heart. [127] Seven of the subunits in this complex are coded by mitochondrial DNA. It has a hydrophilic arm which protrudes into the matrix and a hydrophobic arm (mitochondrially coded) that is embedded in the inner mitochondrial membrane. [128] It has been divided into three functional components: an electron input module (N module), an electron output module (Q module) and a proton translocation module (P module). [129] Rotenone is the most commonly utilised complex I inhibitor. It causes an increase in ROS production. [130] Complex I is also involved in cell apoptosis. [131]

Complex II

Complex II (succinate: ubiquinone oxidoreductase) is a membrane protein complex. The crystal structure of this complex has been delineated from porcine heart. Similar to complex I, it has a hydrophobic membrane component and a hydrophilic membrane component. [132] It is part of the Krebs cycle and links it to the electron transport chain. Malonate and oxaloacetate are inhibitors of complex II. [133]

Complex III

Complex III (ubiquinol-cytochrome *c* oxidoreductase) is made of 10 nuclear coded subunits of the total 11 subunits. [124] Like complex I, it is both an electron and proton transporter. It is also called cytochrome bc_1 complex. It contain 3 protein subunits, one di-haem cytochrome *b*, a cytochrome c_1 and a iron-sulphur protein. [134] Cytochrome *b* is the only component coded by mitchondrial DNA. The inhibitors of this complex are classed into those acting on site N or site P. Antimycin A (used in this thesis, see chapter 2 and 6) works on site N. [135]

Complex IV

Complex IV (Cytochrome c oxidase) is the site of production of water from two protons and half a molecule of oxygen. It is the final acceptor of electrons. In addition, it is a site of proton transfer across the inner mitochondrial membrane. It belongs to a family of haem-copper complexes. [135] Cyanide is an inhibitor of

complex IV and acts on the oxygen binding site. [136]

Complex V

Complex V has two domains. One (F1) is in the mitochondrial matrix and another (F0) is in the inner mitochondrial membrane. [137] F1 comprises of 5 subunits. F0 is made of 6 subunits that include a c-ring and an oligomycin sensitivity conferring protein (OSCP). [138] This is the site of ATP production. The OSCP is the site of action of Oligomycin, a complex V inhibitor (used in this thesis, see chapter 2 and 6).

The functional organisation of the OXPHOS system formed by these complexes is under debate. The presence of supercomplexes have been noted in yeasts as well as mammalian cells. [139] A 'plasticity model' has been suggested as their mechanism of action. According to this theory, the supercomplexes take part in ATP production with different individual component complexes. [140] Supercomplexes are thought to make the electron transport chain more efficient. In addition, they may have a role in controlling the ROS production. [139]

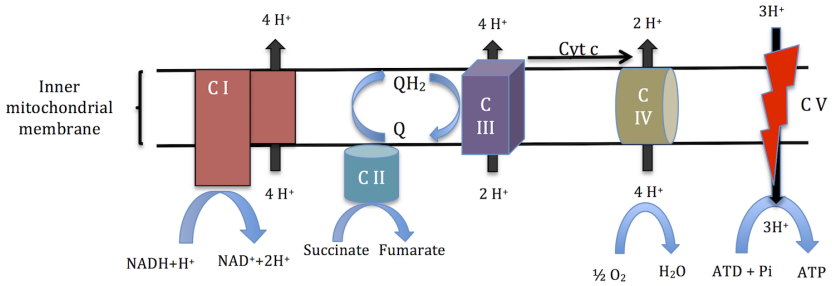
The mitochondrial electron transport chain is the most important source of ROS production. [141] The primary ROS produced by mitochondria are superoxide radical and hydroxyl radical. The hydroxyl radical is the more reactive ROS. Most

of the ROS is formed in complex I. This is due to reverse electron transfer and inhibition of electron transport chain downstream. A small proportion is from complex III. [22]

The overall function of the OXPHOS system is the transfer of electrons from nicotinamide adenine dinucleotide (NAD reduced form) and flavin adenine dinucleotide (FAD reduced form) to oxygen. During this process protons are extruded into the intermembrane space (through complex I, III and IV) from the matrix. Ubiquinone is the intermediate carrier of protons between complex I / complex II and complex III while cytochrome C interacts between complex III and complex IV. This results is an electrochemical gradient across the inner mitochondrial membrane. This proton motive force is utilised when there is a need for ATP production. The protons return to the matrix through the $F_1 - F_0$ rotary mechanism of the ATP synthase causing the phosphorylation of ADP to ATP. [140] [142] (Figure 1.9)The substrates of OXPHOS sytem (NADH and FADH2) are formed in the Krebs cycle. This cycle utilises acetyl Co-A. Most of the primary substrates (pyruvate and fatty acids) are converted to acetyl Co-A in the mitochondrial matrix to facilitate the Krebs cycle. [143]

Figure 1.9: The Oxidative Phosphorylation System

The electrons move along the chain and protons move across the membrane at complex I, III and IV. ATP is formed at complex V. The oxidative phosphorylation system is situated on the inner mitochondrial membrane. Adapted from the Acin-Perez article. [140]



1.12 Mitochondrial Diseases

Mitochondrial diseases are a group of inherited disorders characterised by an impairment in energy production secondary to a genetic mutation leading to a biochemical defect affecting the oxidative phosphorylation pathway. [144] These diseases are rare with an estimated combined incidence of about 1 in 5000 births. [145] The first reported case of mitochondrial disease was a patient with hypermetabolism. [146] This complex heterogeneous group of diseases are difficult to diagnose and no cure exists for the majority of cases. Diagnosis is often challenging because of a lack of specific clinical features. The treatment options available are also limited. Therapeutic strategies are directed at the augmentation of mitochondrial biogenesis, removal of toxic metabolites and to boost the electron transport chain. [147] Riboflavin and Coenzyme Q_{10} are supplements that have been tried

to date. These work in specific subgroups. The vast majority of patient groups derives minimal improvement from these drugs. A recently updated cochrane systematic review noted the lack of convincing evidence for any of the treatment modalities. [148] Currently, these conditions are diagnosed by performing invasive blood, skin and muscle biopsy tests. The 'gold standard' for diagnosis is muscle enzymology studies. Gene mutation analysis is proving useful in diagnosis.

1.12.1 Genetics of Mitochondrial Diseases

Mitochondrial genetics has been defined in detail only in the last 15 years. The 'serial endosymbiont theory' postulates that the mitochondrion was formed after the first eukaryote organism engulfed a prokaryote and formed a symbiotic relationship. The discovery of two genomes (nuclear and mitochondrial) lends support to the above theory. [149]

Both nuclear genes and mitochondrial DNA code for mitochondrial proteins. Children inherit the nuclear genes (20,000 genes) from both parents and it is estimated that about 1500 of these are needed for mitochondrial function. Mitochondrial DNA (37 genes), however, are inherited wholly from the mother. They were discovered in the 1960s. They code for 13 of the >90 proteins in the OXPHOS

system. [150] [151] Mitochondrial DNA (mtDNA) are prone for mutations and reactive oxygen species damage. [152] Mutations in nuclear and mitochondrial genes can cause mitochondrial diseases. About 75-90% of the diseased patients express a mendelian pattern of inheritance due to a mutation in the nuclear genes. The reminder 10-25% are due to mitochondrial mutations. [153] [154] The mitochondrial mutations are either mutations in the genes encoding for mitochondrial synthesis of Ribonucleaic acids, in the electron transport chain proteins or others such as gene deletions (Kearns-Sayre syndome). [155] [156] A very small proportion of mitochondrial diseases are drug induced or due to sporadic mutations. [157] Heteroplasmy adds further complexity. This is the condition wherein within a cell both mutated mtDNA and normal mtDNA are present. The proportion of mutated mtDNA determines the severity of the disease. More than 250 mutations in the mitochondrial DNA have been reported. In addition, over 200 nuclear disease genes have been described. [112] [158]

1.13 My Hypotheses

In conclusion, the available evidence to date suggests that hypoxia is harmful. There is some early evidence that suggests harm from hyperoxia too. However, the safe range for arterial PaO2 is unclear. The risk and benefits of many ICU

interventions is unknown. As most therapeutic strategies in intensive care try to achieve normal physiology, there is an onus to understand the oxygen delivery - consumption balance better during critical illness.

Null hypotheses of my thesis:

Hypoxia and hyperoxia do not cause harm in paediatric critical illness.

Hypoxic adaptation does not occur during paediatric critical illness.

The questions that will need to be answered to disprove the null hypothesis are:

1. Is there evidence that hypoxia or hyperoxia do not have the expected detrimental effect in paediatric critical illness?
2. Does hypoxic adaptation occur in children in hypobaric hypoxic conditions?
3. Is there any evidence of hypoxic adaptation in critically ill children?

In each of the results chapters, I have investigated one step towards my attempt at disproving the null hypothesis.

Chapter 3: Systematic review and two observational studies.

These projects investigate:

1. The evidence of harm from hypoxia and hyperoxia in acutely ill children and

2. Test the hypothesis that there is no relationship between PaO2 and mortality in critically ill children. Both these hypotheses would look for any pattern which might indicate that hypoxic adaptation is at play.

Chapter 4: Utility of NIRS VOT.

I have tested two hypotheses. They are:

1. NIRS VOT is unable to measure oxygen consumption from forearm muscle.

2. NIRS VOT is unable to diagnose mitochondrial disease.

Chapter 5: Oxygen consumption and delivery in hypobaric hypoxic conditions.

The hypotheses tested in this chapter were:

1. Whether or not children can demonstrate adaptation to hypoxic conditions induced by low partial pressure of atmospheric oxygen.

2. Children will have similar cardiorespiratory response as adults when exposed to hypobaric hypoxic conditions.

3. It is safe for children to trek to moderately high altitude (3525 meters).

4. There will not be a change in oxygen consumption during the short time period (4 days) of exposure to hypobaric hypoxic conditions.

Chapter 6: Oxygen consumption and delivery in critically ill children.

The hypotheses tested in this chapter were:

1. Serial measurements of drop Tissue Oxygen Index (TOI) during the vascular occlusion test will not alter during the time course of critical illness in children.

2. Serial measurements of peripheral blood mononuclear cell mitochondrial oxygen consumption will not alter during the time course of critical illness in children.

3. Gene expression of oxidative phosphorylation genes will not change in the first 48 hours of paediatric critical illness.

Chapter 2

Methods

2.1 Near Infrared Spectroscopy with a Vascular Occlusion Test

The Near infrared spectroscopy with a vascular occlusion test (NIRS VOT) has been proposed as a tool to estimate dynamic changes in oxygen consumption in forearm muscle. The test involves a continuous measure of tissue oxygen index (TOI) and incorporates a period of arterial occlusion followed by recovery. The post occlusion phase reflects post ischaemic reperfusion / hypereamia. The technique has been utilised in septic shock patients and patients under anaesthesia. [159] The NIRS VOT technique has been employed in two formats: a 'time-targeted' VOT and a 'TOI-targeted' VOT. The former format involves inflating

the blood pressure cuff for 3 minutes whilst the later format continues the cuff inflation until the TOI value decreases to 40%. I chose the time-targeted format, as this was considered more tolerable for children. [160] [161] The phases of NIRS VOT are: baseline, downslope (from the start of arterial occlusion to release), upslope (immediately follows release of occlusion) and recovery. (Figure 2.1)

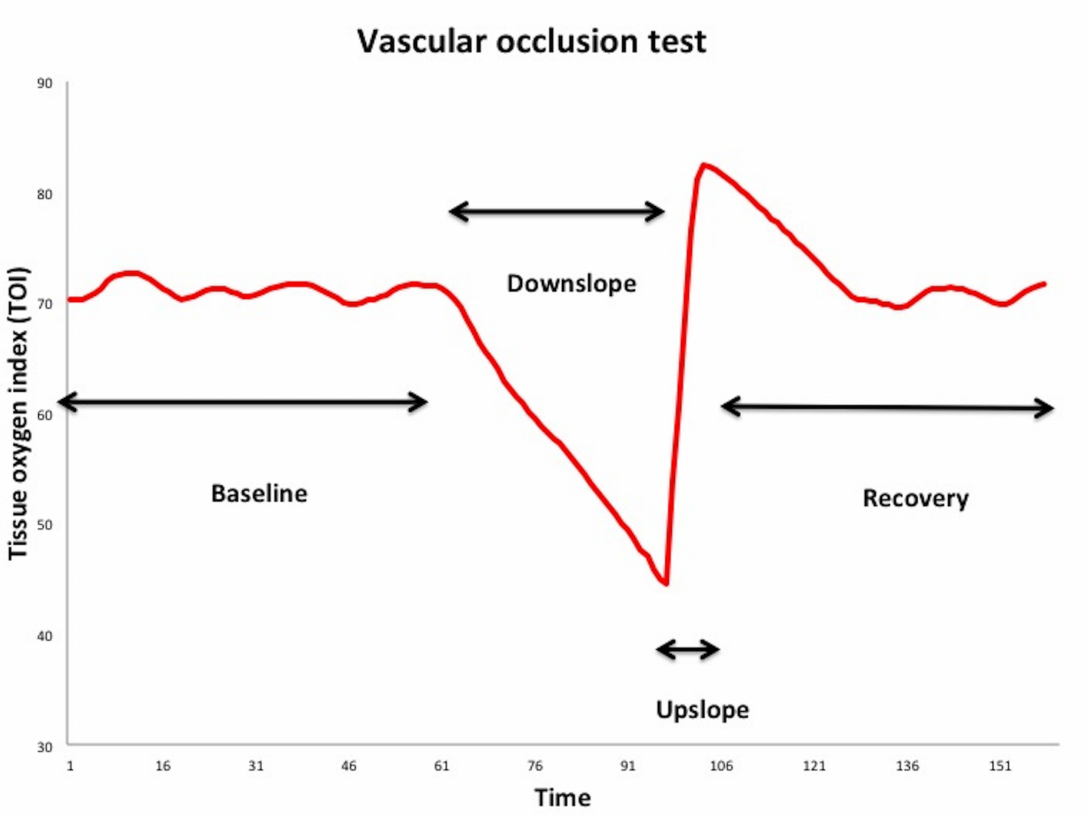
Figure 2.1 is an example recording of a vascular occlusion test showing baseline, downslope, upslope and recovery phases. The blood pressure cuff was inflated at the end of baseline phase. The deflation of the blood pressure cuff denoted the end of downslope and the start of upslope. The NIRS VOT measures the Tissue Oxygen Index (TOI). The DropTOI is the difference between the lowest TOI (at the end of cuff inflation) and baseline TOI.

The baseline TOI is a marker of the forearm oxygen saturation at rest. The downslope is a surrogate for forearm oxygen consumption. The DropTOI is the difference between lowest TOI and baseline TOI. The DropTOI is of particular relevance to my hypothesis of hypoxic adaptation. A lower DropTOI (i.e. downslope with a less steep gradient) indicates reduced oxygen utilisation. This change would be expected in the event of successful hypoxic adaptation. The upslope is due to post occlusive hyperaemia from reactive vasodilation. This phase has been

used as to assess microcirculation. When the upslope is has a slower gradient, it might suggest delayed vasodilation. This can be due to pre-existing maximal vasoconstriction (high sympathetic activity) or vasoplegia. Recovery phase is the final normalisation phase. The downslope and recovery phase vary with different clinical states such as sepsis and post-trauma. [162]

Figure 2.1: The Near Infrared Spectroscopy Vascular Occlusion Test Curve

The x-axis denotes time in a compressed scale. The y-axis shows the tissue oxygen index as recorded by the near infrared spectroscopy machine. The horizontal black lines separate the recording into 4 phases. The inflation of the blood pressure cuff denotes the end of baseline (1) and start of the downslope (2). The downslope ends at the deflation of the cuff. This is followed by upslope (3) and then recovery phase (4).



Several adult studies have indicated that NIRS VOT may be suitable methodology for use in critically ill patients. This technique is able to differentiate between patients with and without a high lactate. Patients with poor perfusion and a high lactate were observed to have a lower downslope with a vascular occlusion

test. This finding suggests that in the setting of decreased oxygen delivery (lack of oxygen), tissues have decreased oxygen consumption and anaerobic pathways that produce ATP are active. [163] Patients in septic shock showed a similar decrease in downslope. This finding could be attributed to a mechanism similar to the above study i.e. decreased oxygen consumption. [164] In post-trauma patients, the upslope was noted to be lower. As this study had a heterogeneous trauma population, it is difficult to draw firm conclusions from this finding. One might hypothesise that there was an element of vasoplegia in these patients. It would be interesting to explore how many of these were spinal injury patients. This was not reported. [165] In a systematic review that included 20 studies, drop TOI (difference between lowest TOI and baseline TOI) was observed to be lower in septic patients compared to healthy controls (7.8% vs. 12.5%, p = 0.01) suggesting lower oxygen consumption. [166] In a recent adult study, serial NIRS VOT was performed in critically ill patients (n= 89) and healthy controls (n=27). They demonstrated slower desaturation in the last phase of downslope in patients. The drop TOI was higher in non-survivors everyday until day 3 of ICU admission. The lower baseline TOI was observed with sepsis, high lactate, use of noradrenaline and hypotension. A few points are worth discussing further regarding these findings. First, there was slower desaturation in patients. This suggests lower oxygen consumption. Interestingly, the desaturation was slower in the last phase of the

downslope. This might be due to an impaired microcirculatory autoregulation and inability to redirect blood flow (augment oxygen delivery) during ischaemic challenge. The second finding was that non-survivors seemed to have higher oxygen consumption. This might be interpreted as an inability to adapt adequately to hypoxic conditions. Finally, the lower baseline probably demonstrates the existing haemodynamic instability and decrease in oxygen delivery in this critically ill population. [167]

The above studies suggest that NIRS VOT measures the decreased oxygen consumption in critically ill patients. Further, in some populations (trauma), NIRS VOT enables assessment of the microcirculatory autoregulation. In addition, there maybe an association between drop TOI and ICU outcome. These findings indicate that NIRS VOT might be a suitable technique to assess dynamic oxygen consumption in critical illness.

The vascular occlusion test (VOT) acts as an internal calibration within each patient. This is because both oxygenated Hb and deoxygenated Hb reach near zero values at different points of the test. The NIRO-NX 200 manufacturers' guide also suggests that calibration is not necessary. [168]

One part of basic physiology that might impact the results is the effect of vasoconstriction. Lima showed significant variation of TOI with peripheral vasoconstriction due to surface cooling in healthy volunteers. [169] The same group assessed the variation of TOI in critically ill patients. They demonstrated an influence of vasopressors and the status of peripheral circulation on the recovery phase of NIRS VOT. Interestingly, they were unable to detect an impact of any of these on the downslope of NIRS VOT. [170] This finding is particularly relevant for my thesis. The limitation of NIRS VOT in this context might the lack of specificity of drop TOI. The drop TOI may be affected by mitochondrial changes or microvascular dysfunction (secondary to pathophysiology or therapeutic vasoactive drug use).

The NIRS VOT was part of three projects within this thesis: Young Everest Study 2 (healthy children who trekked to Namche Bazaar - 3525 metres), children with a suspected mitochondrial disease, and children admitted to the intensive care unit. The test lasted for 13 minutes. Subjects lay down on a comfortable bed. They rested their forearms on pillows. On each forearm, two 2 cm probes were placed on the skin overlying the Brachioradialis muscle (upper lateral aspect of the forearm). To secure the probes to the forearm, probes were wrapped with a bandage. The bandage also reduced interference from ambient light. The data were displayed as a continuous graph and represented a change in the tissue oxygen index. This

was the baseline tissue oxygen index measurement. An appropriately sized blood pressure cuff was then placed on the non-dominant arm. After a 5 minute recording of the baseline tissue oxygen index, the blood pressure cuff was inflated. This was maintained at 30 mm Hg above systolic blood pressure for 3 minutes and then deflated. A further 5 minutes of recording were measured. This concluded the test. Figure 2.1 shows a healthy volunteer undergoing a VOT.

Figure 2.2: Healthy Volunteer Undergoing a Vascular Occlusion Test

The volunteer lay down comfortably. The probes were placed on the forearm and wrapped with a bandage. The volunteer also had a saturation probe on the index finger and a blood pressure cuff on the arm. After a 5-minute baseline measurement, the blood pressure cuff was inflated for 3 minutes. A further 5-minute recording was made after deflating the cuff.

2.2 Cardiac Output Monitoring

Cardiac index was measured in all subjects using an Ultrasound Cardiac Output Monitor (USCOM). The height, weight, systolic and diastolic blood pressure were

also collected for calculating this measure. The subject lay down comfortably on the bed. A small quantity of ultrasound gel was applied to the lower part of the neck. The USCOM probe was placed on the suprasternal notch and directed towards the apex of the heart. The aim was to achieve a waveform with a triangular apex. Calculation of the cardiac index was from the sum of a group of waves. The recording was saved over a 2 minute period. This was repeated three times. The average gave a measure of cardiac index.

Physiological parameters that might have an impact on the cardiac index measurement such as heart rate, temperature, inotropes being administered, fluid resuscitation, and the aetiology of illness were also recorded where available. Some of these measurements were only possible for the children in the intensive care unit. Figures 2.3 and 2.4 illustrate the direction of probe placement and measurement in a volunteer. [70]

Figure 2.3: Direction of Probe Placement for Aortic Flow Measurement

The tip of the probe is directed towards the apex of the heart. The aortic valve lies in this direction. The Doppler wave hits the blood exiting the valve and returns to the probe. Reproduced from the USCOM product literature by Phillips. [70]

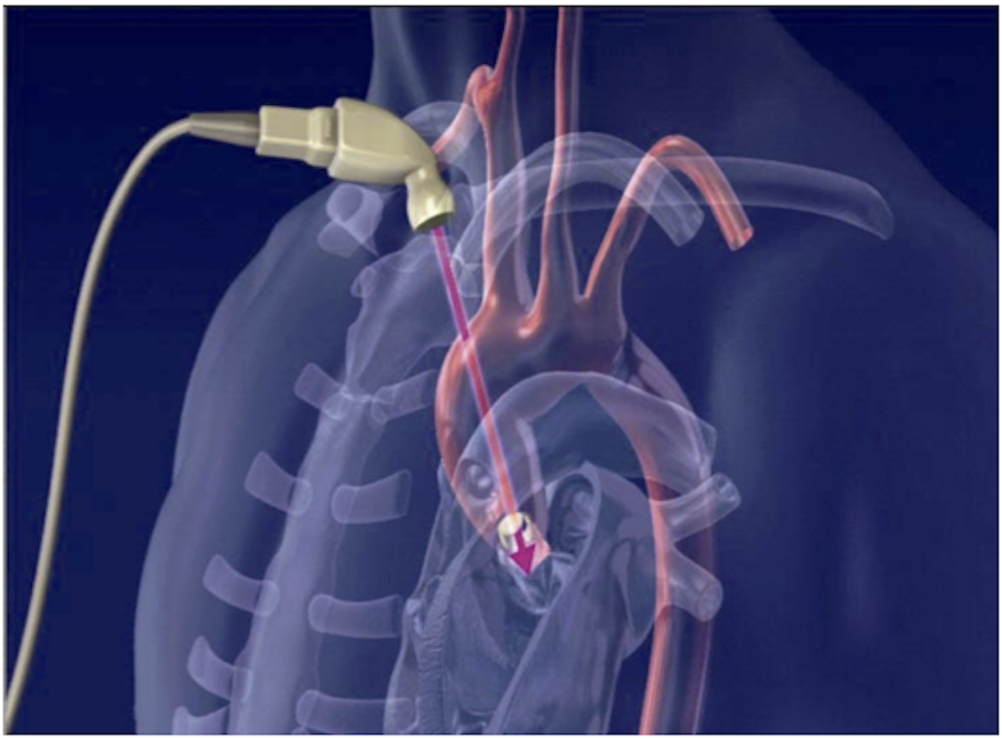


Figure 2.4: Healthy Volunteer Undergoing USCOM Measurement

The volunteer is lying flat on the bed with head in midline. The probe is placed on the sternal notch and directed towards the left nipple. Reproduced from the USCOM product literature by Phillips. [70]

2.3 Oxygen Consumption in Peripheral Blood Mononuclear Cells

2.3.1 Oroboros High Resolution Respirometry

The Oxygraph-2K high resolution respirometry analyser (O2K, Oroboros instruments, Innsbruck, Austria) measures the rate of oxygen consumption in cell suspensions. The O2K analyser has an airtight chamber with an oxygen electrode in a solution. The oxygen consumption is deduced from the change in concentration

of oxygen in the chamber after instilling permeabilized or intact cells and reagents. The analyser was first developed in the late 1990s. [171] A step-wise titration of reagents can be performed with this analyser. In addition, each step of the electron transport chain can be interrogated.

This analyser was selected since it has been shown to give reproducible results. Furthermore, it enables interrogation of oxygen consumption from several tissues. Tissues that may be interrogated with this analyser include muscle fibers, peripheral blood mononuclear cells and platelets. [172] [173] [174] [175] It was utilised in the adult Xtreme Everest studies. The O2K machines were transported to the Everest base camp to analyse skeletal muscle oxygen consumption. This proved its robustness. [176]

I undertook a 5-day on-site training in Schroeken, Austria to learn the O2K methodology. For my experiment, I hired the O2K machine situated in Starling lab, UCL. The daily machine calibration was run by Dr Kotiadis (post doctoral research associate). An air calibration was performed before each run of the experiment by me.

The following reagents were used in my experiment.

1. Oligomycin - This is a complex V inhibitor (ATP synthase of the oxidative phosphorylation system). This causes leak respiration.

2. FCCP (Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone) - This is a protonophore and acts as an uncoupler. It dissociates the proton concentration outside the mitochondrial membrane and electron transport and oxygen uptake. In turn it separated the ATP synthase from the electron transport chain. Therefore, it causes maximal respiration (not associated with ATP production) that is termed Electron Transport System capacity (ETS).

3. Antimycin A - This is a complex III inhibitor. It causes residual oxygen consumption by fully inhibiting the oxidative phosphorylation system.

The reagents were sourced from Sigma-Aldrich industries. Oligomycin was in the powdered form of 'Oligomycin from Streptomyces diastatochromogenes'. FCCP was in the powdered form. Antimycin was in the powdered form of 'Antimycin A from Streptomyces species'. All three reagents were reconstituted as detailed below.

Preparation of reagents

1. Oligomycin: To make 5mM solution.

a. Weigh 4 mg Oligomycin in 2 ml glass vial.

b. Dissolve in 0.975 ml 99.9% Ethanol.

c. Divide into 0.2 ml portions and store at $-20\,^{\circ}\mathrm{C}$.

2. Antimycin-A: To make 5mM solution.

a. Weigh 5.4 mg of Antimycin A into a glass vial.

b. Dissolve in 1.85 ml 99.9% Ethanol.

c. Divide into 0.2 ml vials and store at $-20\,^{\circ}$C.

3. 4-(trifluoromethoxy)phenylhydrazone (FCCP): To make 1mM solution.

a. Weigh 1.27 mg of FCCP into a glass vial.

b. Dissolve in 5 ml 99.9% Ethanol.

c. Divide into 0.2 ml portions and store at $-20\,^{\circ}$C.

Table 2.1: Final concentration of the stock solution of the reagents

Reagent	**Titration volume**	**Final concentration**
Oligomycin	1 μL	2 μmolar
Antimycin-A	1 μL	2.5 μmolar
FCCP	1 μL	0.5 μmolar

Key: FCCP - 4-(trifluoromethoxy) phenylhydrazone

2.3.2 Phases of Respiration

The different phases of respiration that I assessed as part of my experiment were (Figure 2.6):

1. Routine respiration (R) - This is the respiration (molecules of oxygen utilised) by the peripheral blood mononuclear cells without the addition of any reagents. It represents the aerobic metabolic activity under routine culture conditions with the physiological substrates in the RPMI medium. It depends on cellular energy demand, energy turnover and the degree of coupling to phosphorylation.

2. Leak respiration (L) - Under normal conditions, electrons flow along the electron transport chain and protons are extruded across the inner mitochondrial membrane from the matrix to the inter-membrane space. When ATP synthase is inhibited, by adding Oligomycin to the solution, no ATP is produced. However, as the flow of electrons and protons continue to occur, a proton gradient is generated across the inner mitochondrial membrane. This is called the proton motive force. At maximal proton motive force, some protons passively leak back into the matrix of the mitochondria. This is called proton leak. It is the dissipative component of respiration and is not available for basal metabolic requirements. This is represented as leak respiration in O2K high resolution respirometry.

3. Residual oxygen consumption (Rox) - Some oxygen is utilised by cell pathways other than the electron transport chain. This is estimated by assessing oxygen utilization after inhibition of oxidative phosphorylation by inhibition of complex III by Antimycin A.

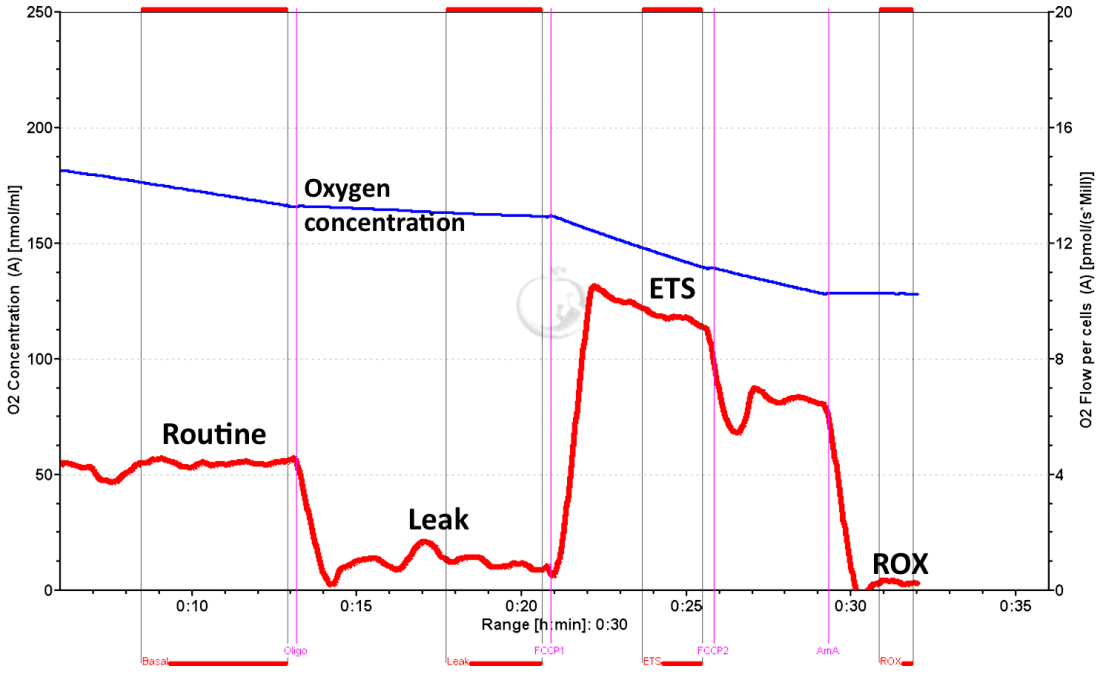
All of the above values (routine, leak and residual oxygen consumption) are measured in $picomols.sec^{-1}.10^{-6}cells$. Figure 2.5 shows the steps of oxidative phosphorylation with the sites of action of the three reagents utilised in my experiment.

Figure 2.5: Steps of Oxidative Phosphorylation Depicting the Sites of Action of the Reagents Utilised in the Oroboros Analysis

The Tricarboxylic acid (TCA) cycle (also called Krebs cycle) provides substrates to complex I and II. Electrons move along the system to Q-junction. Protons are extruded to the cytosol (inter-membrane space) from the matrix. Complex III receives electrons from Q-junction. Antimycin A blocks complex III. The next step is Complex IV through cytochrome C. Adenosine Triphophate (ATP) synthase utilises the proton gradient across the mitochondrial membrane to form ATP from Adenosine diphosphate (ADP). Oligomycin blocks this function and stops ATP production. Some protons leak across the membrane. Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP - Mitochondrial uncoupler) is added as sequential titrations. This is a protonophore and causes the dissociation of the electron transport system and ATP production.

Figure 2.6: Example of a Typical Output from Oroboros Analyser

The primary y-axis denotes oxygen concentration in the chamber. The blue curve represents decreasing oxygen concentration. The secondary y-axis denotes oxygen flow per cell. This is presented as the red curve, which depicts the oxygen consumption during the experiment. The x-axis denotes time (minutes). The vertical boxes with red borders represent the periods that were utilised for summative measurement. The first box is for basal respiration. Oligomycin A (ATP synthase inhibitor) was added which leads to leak respiration. Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP - Mitochondrial uncoupler) is added as sequential titrations. This gives the Electron Transfer System capacity value (ETS). The last reagent added is Antimycin-A (Complex III inhibitor) which abolishes respiratory chain function completely, leaving what is known as the Residual Oxygen Consumption (ROX)



Before discussing the control ratios I employed in my experiments, let me briefly discuss coupling and uncoupling in mitochondrial physiology. Under normal conditions, the electron transport chain and ATP synthase are coupled forming the

oxidative phosphorylation system. The ATP is formed as an end product due to the coupling of the electrochemical system (transfer of electron and formation of a proton gradient) to phosphorylation system (ADP to ATP). Despite the leak of some protons back into the inter-membrane space, coupling ensures efficient utilisation of substrates and oxygen to produce energy.

Uncoupling of the electron transport chain and ATP synthase can occur in certain situations such as membrane damage and activation of uncoupling proteins (UCP). When UCPs are activated, protons leak back into the inter-membrane space without the production of ATP. The main role of UCPs is to control excessive production of reactive oxygen species (ROS). The UCP2 and UCP3 are stimulated by ROS. These in turn, cause a negative feedback loop and limit ROS production. [177] They are also involved in thermogenesis (UCP1) and insulin secretion (UCP2). Their gene expression is tightly linked to the need for heat production or regulation of ROS production. [18]

2.3.3 Flux Control Ratios

Both routine and leak respiration vary according to the mitochondrial content. In addition, different tissues from the same individual may also show varying

respirometry measurements due to mitochondrial heterogeneity. Consequently, the respiration data have to be normalised for the mitochondrial content to enable inter and intra-subject comparison. Surrogate markers such as cardiolipin and citrate synthase have been used to derive the mitochondrial content. [81] Ideally I would have used a citrate synthase assay to normalize for mitochondrial content: unfortunately this was restricted by time/resource availability.

In the absence of a measure of mitochondrial content, Flux Control Ratios (FCR) are the standard way to express oxygen consumption independent of mitochondrial content (total volume of mitochondria). [178] This normalization procedure calculates the oxygen consumption during leak and routine respiration as a proportion of the maximal oxygen consumption. The maximal oxygen consumption can be elicited in intact cells by administering FCCP. This is termed Electron Transport System (ETS) capacity. By normalizing for ETS, the ratios enabled like-for-like comparisons between sequential daily measurements of respiration states within the same subject. These were particularly relevant in my study in order to normalise for the mitochondrial heterogeneity.

The FCR includes routine control ratio, net routine control ratio, leak control ratio and coupling efficiency. These are defined as the oxygen flux or consumption

during different respiratory states - such as routine or leak respiration - normalized for maximum oxygen consumption after administration of FCCP (ETS capacity). The lower and upper limits of the ratios are 0.0 and 1.0. [179]

Figure 2.6 depicts the periods of routine respiration (R), leak respiration (L), ETS capacity and residual oxygen consumption (Rox). I will be utilising the following measures to analyse the results of my O2K experiments.

1. Leak control ratio (LCR - L/E): This is the ratio of leak respiration (L) *and* ETS capacity (E - maximum FCCP stimulated oxygen consumption). The L/E ranges from 0.09 to 0.14 in human cells. [179]

The L/E ratio could be a measure of uncoupling. Uncoupling increases the L/E ratio by increasing the leak respiration. The L/E ratio can also increase if the ETS capacity is reduced. Ideally, therefore, defects of ETS capacity should be ruled out by measuring ETS capacity per mitochondrial marker (such as citrate synthase). [180]

It has been hypothesised that during hypoxic adaptation, uncoupling is reduced in preference to a more efficient coupled respiration. [181] In-vivo studies have demonstrated a decrease in LCR in human islet cells exposed to hypoxia. [182]

In addition, hypoxic mice were observed to have a decrease in mitochondrial biogenesis and uncoupling protein levels. [183] Finally, human skeletal muscle after exposure to hypobaric hypoxic conditions were noted to have decreased mitochondrial biogenesis. [176] Following on from the above studies, in my O2K study, a drop in L/E ratio during recovery from critical illness might be a sign of hypoxic adaptation.

2. Net Routine Control Ratio [net RCR - $(R-L)/E$]: Net RCR is the difference between routine (R) *and* leak (L) respiration as a proportion of maximal FCCP stimulated oxygen consumption (E- ETS capacity). In other words, it is the oxygen consumption due to phosphorylation after normalizing for leak respiration and ETS capacity. Under normal conditions, 0.1 to 0.3 of ETS capacity is used for oxidative phosphorylation. [184]

In a septic child with an increased ATP demand, this ratio increases showing that the ATP formation increases due to an increase in routine respiration. When the child is in a stable compensated state, this ratio remains steady. When there is full uncoupling, this ratio becomes zero. [179] [185]

For the purpose of my O2K study, net RCR gives an indication of the proportion of oxygen utililsed to produce ATP. With hypoxic adaptation, I hypothesise that the net RCR increases.

3. Coupling efficiency $[(E-L)/E]$: Coupling efficiency is the difference between maximal FCCP stimulated respiration (E-ETS capacity) *and* leak respiration (L) as a proportion of maximal FCCP stimulated respiration (E-ETS capacity). In other words, it is the amount of coupling occurring when normalised for leak respiration. [184]

As mitochondria become more efficient with hypoxic adaptation, leak respiration decreases. This results in an increase in coupling efficiency. This has been demonstrated in healthy volunteers after acclimatisation to hypobaric hypoxic conditions. [186]

In my O2K study, if hypoxic adaptation were to occur, I would expect an increase in coupling efficiency with recovery from critical illness.

With the limitations of the O2K technique, the ratios detailed above might be the best method available to investigate the mechanism of hypoxic adaptation. For example, L/E ratio decreases with increasing duration in hypoxic conditions whilst coupling efficiency increases under these conditions. As this is a novel methodology, no other ratios have been investigated for hypoxic adaptation assessment. [187]

Therefore, if I were to demonstrate these changes in critically ill children it might support my hypothesis that hypoxic adaptation occurs during recovery from critical illness.

2.3.4 Protocol

The Oxygraph-2K analyser measures oxygen consumption from cells suspended in a solution. The analysis involved two steps: isolation of peripheral blood mononuclear cells from whole blood and analysis.

Peripheral Blood Mono-nuclear Cells (PBMC) separation protocol:

Blood sample collection:

1. 5 μL heparin was pippetted into a 20 ml Falcon tube. The tube was inverted to ensure heparin coated the sides of the tube.

2. 5 ml of blood was collected in a syringe and transferred immediately into the Falcon tube precoated with heparin (Falcon tube 1). The sample was mixed well. A blood sample was collected every day whilst the patient was in the intensive care unit.

Ficoll-Paque density gradient cell separation of PBMCs:

1. The Falcon tube 1 was topped up with plain RPMI medium (Roswell Park Memorial Institute) in a 1:1 proportion (5 mls).

2. In new 2 ml Falcon tube 5 ml of Lymphoprep was collected. This was carefully

overlaid with the contents of Falcon tube 1 (diluted blood with RPMI) at an angle (Falcon tube 2).

3. The contents of Falcon tube 2 were spun at 2400 RPM for 20 minutes without brakes or acceleration at room temperature (Temperature set at 25 °C).

4. The PBMCs were harvested with a sterile 3 ml Pasteur pipette into a new 20 ml Falcon tube (Falcon tube 3). Three mls of the separated serum was collected and stored in −80 °C freezer.

5. The Falcon tube 3 contents were topped up with plain RPMI. This was spun at 1800 RPM for 10 min without brakes.

6. The supernatant was discarded. The pellet was re-suspended in 2.6 mls of the plain RPMI medium. A 0.2 ml aliquot of this cell suspension was collected in an Eppendorf tube and stored in −80 °C freezer for later citrate synthase assay. A further 0.2 mls of the suspension was taken to the haematology lab in another Eppendorf tube for Fluorescence Activated Cell Sorting (FACS) analysis.

The final volume of 2.2 mls of cell suspension containing PBMCs was transported to the Starling lab.

Previously employed O2K techniques were utilised to measure the oxygen consumption in peripheral blood mononuclear cells. [172] This included daily sampling of blood (5 mls) soon after the NIRS VOT for the recruited patients.

Oxygraph-2k experiment protocol

Set up:

Temperature 37 °C

Volume 2.2 ml / chamber

Stirrer speed 750 rpm

Data sampling interval 2 s

Concentration: 1 million cells / ml. No viability check was performed as ratios were employed.

Sensor calibration and Instrumental calibration:

The chamber was filled with 70% Ethanol for 20 mins for sterilization. The chamber was then washed three times with distilled water and filled with plain RPMI medium.

Air calibration:

a. Ensure gas phase above the medium

b. Wait for signal stabilisation

c. Mark R1 as the period of stable signal

d. Open datlab calibration window. Input temperature (37 °C) and solubility of medium (RPMI 0.89)

e. Click calibrate and copy to clipboard

f. Save file on calibration excel sheet for both chambers separately

Instrumental background calibration would have been done in the morning.

Experiment:

1. Ensure stirrer is on and the laptop is connected to the machine

2. Add 2.2 mls of cell suspension into the chamber

3. Stir cell suspension for 2 minutes in contact with air for oxygenation

4. Close chamber. Wait for 5 minutes for signal stabilisation

5. Start titrations:

a. Record 10 mins of Basal respiration (B)

b. Add Oligomycin A (1 microL; 2 microg/ml = 2 microMolar). Record Leak respiration (L) for 5 mins

c. Add Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (Each titration: 2 microL; 1 microMolar). Record maximal respiratory capacity (ETS). Add 1 -2 more titrations of the same volume of FCCP. Notice the inhibition of respiration with excess uncoupler. This recording takes about 10 minutes.

d. Add Antimycin A (1 microL; 2.5 microM). Record the residual oxygen consumption for 10 minutes.

The experiment ended and the recording was saved in my folder. Finally, the

chamber was washed three times with 100% ethanol, three times with distilled water and then left with 70% Ethanol in the chamber for the night.

2.4 Data Handling

The USCOM, NIRO-NX monitor and Oxygraph-2K give outputs in an excel format. All recordings and the excel outputs were saved in an University College London computer and Great Ormond Street Hospital computer. Serial study numbers were used for anonymisation of the data. This followed format: Subject 1 (Metabolic disorder group)- GM101, Subject 1 (Intensive care group) - GICS101, Subject 1 (Young Everest Study 2) - Y01, SOCK101 - Serial oxygen consumption study.

A Great Ormond Street Hospital computer holds a master file with codes for the specific patients. This linked anonymisation is necessary to correlate the NIRS data with the results of all clinical investigations performed as part of the patients management.

Physiological data were recorded in the REDCAP database. REDCAP is a secure database created by the Vanderbilt University. [188]

Chapter 3

Prevalence and Outcome of Hypoxia and Hyperoxia in Critically Ill Children: A Cohort Study

This chapter addresses the relationship between arterial oxygenation and mortality in critically ill children. First, I set out to study the PaO_2-Mortality relationship internationally. To achieve this, I completed a systematic review that investigated the association of admission arterial oxygenation with survival in children.

Next, I set out to describe the incidence of hypoxia and the relationship between

admission PaO_2 and mortality in the paediatric intensive care unit (PICU) in Great Ormond Street Hospital (GOSH). I performed this observational study in two parts: a threshold study ('extreme hypoxia') and an association study assessing relationship of PaO_2 (as a continuous variable) to mortality.

Finally, I aimed to ascertain if practice on the PICU in GOSH is representative of that which is followed in other units across the United Kingdom. To this end, I conducted a national survey exploring the decisions in administering supplemental oxygen in all PICUs in the country. This survey was conducted to explore if a clinical trial of 'restrictive' to 'liberal' oxygen strategy was justified.

3.1 Introduction

The administration of supplemental oxygen is widespread in the management of critically ill children. The aim of this therapy is to augment oxygen delivery to the tissues (DO_2). An increase in partial pressure of arterial oxygen (PaO_2) should in the normal physiological state increase DO_2. The eventual effect of the increase in DO_2 on tissues is unclear. As alluded to in chapter 1 (section1.3), high oxygen concentration in the cells can lead to the production of reactive oxygen species (ROS). Reactive oxygen species, in turn, have a potential to cause cell injury. Conversely, low oxygen levels (hypoxia) cause cell death. Therefore, the risks and

benefits of oxygen therapy need to be evaluated by the clinician.

Do clinicians consider oxygen toxicity when devising management strategies in the intensive care setting? Eastwood et al surveyed the practice of a large cohort of intensivists in Australia and New Zealand. Of a total of 99 respondents, 77% reported prescribing oxygen saturation targets. Clinicians working in a regional centre were less concerned with oxygen toxicity. Further, only 26.5% of the respondents considered oxygen related lung injury to be a major concern. [189]

A Dutch study explored the response of clinicians to arterial blood gas values with a retrospective database analysis over a 5-year period in tertiary intensive care units. They interrogated 126,778 arterial blood gas values by setting PaO_2 >16 kPa as the threshold for hyperoxia. Although 28,222 PaO_2 values (22%) were higher than the threshold, clinicians reduced the FiO_2 in only 25% of these situations. [190] Other than individual clinician's practices, a recent survey reports that three-quarters of adult intensive care units do not have a strategy for the management of refractory hypoxaemia. [191] This is evidence in adults. Children have important physiological differences.

Multi-organ failure (MOF) is noted in a majority of patients preceding death. [192] In adults, MOF is usually seen by day 3-4 after admission to the intensive care unit. The risk of developing MOF in adults increases with pre-existing chronic disease and lifestyle-related conditions such as obesity, atherosclerosis, malnutrition or al-

coholism. [193] In contrast, MOF occurs earlier (2-3 days) in children. [194] [195] The risks for developing MOF are also not clearly defined in children. This difference in MOF epidemiology extrapolates to survival. Children have better survival compared to adults. The co-morbidities observed in adults only explain this variation in part. The growth and regenerative potential of organs probably present a survival advantage in children. [196]

These differences necessitate a systematic review, one observational study (with two parts) and a national survey.

3.2 Systematic Review

Impact of hypoxia and hyperoxia on survival in unwell children

Harm from both hypoxia and hyperoxia in adult critical illness has been reported. [17] This perceived harm has not yet been investigated in children. There is no consensus for the 'ideal arterial oxygen tension' target in the critically ill child.

The aim was to explore the effect of hypoxia and hyperoxia on mortality in unwell children. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines was abided by for the following report. [197]

3.2.1 Inclusion Criteria

1. Patient: Children from 4 weeks to less than 18 years of age

2. Intervention - Observational cohort:

Hypoxia was defined as peripheral oxygen saturations <90% or PaO_2 <60 mmHg (8 kPa) and

Hyperoxia was defined as PaO_2 >300 mmHg (40 kPa)

For the alternative threshold: Hyperoxia was defined as PaO_2 >200 mmHg (26.6 kPa) and hypoxia was defined as peripheral oxygen saturations <92%.

3. Comparison cohort: Normoxia was defined as PaO_2 between 60-300 mmHg (8.1-40 kPa) or peripheral oxygen saturations >90%.

4. Outcome: In-hospital mortality

5. Study design: Randomised controlled trials and cohort studies

Hypoxia is prevalent in unwell children presenting to resource-limited settings. [198] Inclusion of the hypoxia group in this review enabled comparison of the effect of hypoxia with and without aggressive intensive care practices.

Studies in pre-term infants and neonates with hypoxic-ischemic encephalopathy were excluded because the transition during the perinatal period from a hypoxic to an oxygen rich environment is complex and differs from the pathophysiology of hypoxic injury in older infants and children. [199]

3.2.2 Search Strategy

Randomised controlled trials, cohort studies and observational studies were identified by using predefined search terms in MEDLINE (1950 - Jan 2015), EMBASE (1980 - Jan 2015), Cochrane systematic reviews and DARE databases.

The search terms used in the MEDLINE database were hyperoxia, hypoxia, survival and critically ill children or pediatric intensive care and mortality. The search was conducted in April 2015. The full search strategy in MEDLINE and a snapshot of the EMBASE search is included as appendix2.

3.2.3 Selection Criteria

The principal summary measure was the odds ratio for survival. After initial search and discarding duplicate publications, studies were ascertained by perusing the titles and abstracts.

The risk of bias within individual studies was ascertained by using the Newcastle Ottawa Scale at the study level. [200] A meta-analysis and meta-regression was performed using the OpenMeta[Analyst] software. [201] Meta-regression enabled us to determine the effect of co-variate (Newcastle Ottawa Scale) on the effect estimate (Odds ratio of survival with hyperoxia). For heterogeneity analysis, I^2 was used as the measure of consistency. A priori subgroup analysis with aetiology as the moderator was planned. Creating a funnel plot aided in assessing the risk

of bias across studies. R software was utilised to draw these plots.

3.2.4 Meta-analysis

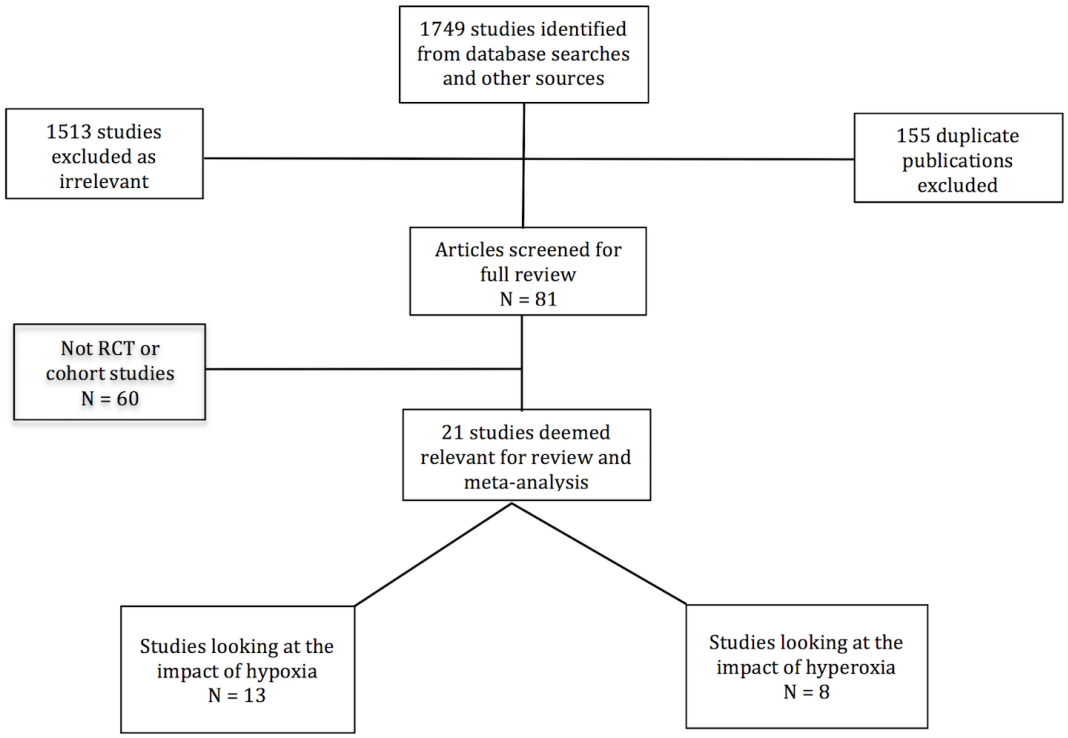
A meta-analysis was performed using a random effects model with OpenMeta[Analyst] software. I^2 was used as the measure of consistency for heterogeneity analysis. For subgroup analysis, the studies were divided into patients admitted with lower respiratory tract infection (LRTI) or CA or TBI.

3.2.5 Results

The initial search strategy resulted in 1749 articles. Duplicate publications (155) were discarded. From the remaining 1594 articles, 1513 were excluded as not being relevant to the current review. For the full review, 81 full-text articles were chosen. Finally, 20 articles and a conference abstract were included for review. The review of the impact of hyperoxia included 6 studies and the hypoxia review consisted of 11 articles. There were 2 further hyperoxia and 2 hypoxia articles deemed relevant when the alternative threshold was employed. The flowchart describes exclusions at each stage. All the articles were cohort studies apart from one with a single centre unmatched case-control design. Experts in the field were contacted to discover any unpublished work.

Figure 3.1: Flow Chart Showing the Selection of the Studies for the Systematic Review

Studies selected after employing an alternate threshold were included in the flowchart.



The hyperoxia studies analysed were: 5 cohort studies of CA and 1 study in children with TBI. The hypoxia studies analysed were: 4 cohort studies in CA survivors, 3 in children with LRTI, 2 studies in children with TBI and one each of children with malaria and diarrhoea.

With the alternative threshold, hyperoxia studies included 6 in CA survivors and 2 in TBI group. The hypoxia studies consist of 4 each in the CA and LRTI groups,

3 with TBI and one each with malaria and diarrhoea.

Analysis of Bias

Publication bias might be present as the range of PaO_2 explored in all the studies were <8 kPa or >40 kPa. The investigators may have considered PaO_2 between 8 and 40 kPa as an extended normal range. Therefore, there are no publications of studies where the relationship to mortality for this range has been assessed. This might suggest a bias in establishing the inclusion criteria for the cohorts. Selection bias was ascertained by reviewing the methodology of studies. As most of them are cohort studies, there was a high probability of selection bias. The risk of bias was only assessed at the study level and not at the outcome level.

The funnel plots were created with the x-axis as log odds ratio and the y-axis as 1/standard error. There might be publication bias in the hyperoxia studies suggested by one of the studies lying outside the 95% confidence interval limits. Even within the plot, there were more studies on the left, suggesting a negative bias.(Figure 3.10) For the hypoxia studies, a single study was outside the 95% confidence interval limits suggesting some bias.(Figure 3.11)

Figure 3.2: Funnel Plot of Hyperoxia Studies Suggesting Publication Bias

The odds ratio and standard error for each of the studies was calculated. The x-axis represents log of the odds ratio (OR). The y-axis denotes one over the standard error (SE). Un-shaded circles represent individual studies. The vertical dashed central line depicts the mean log odds ratio. The solid vertical curved lines on each side of the dashed lines represent 95% confidence intervals. The plot was generated using R software.

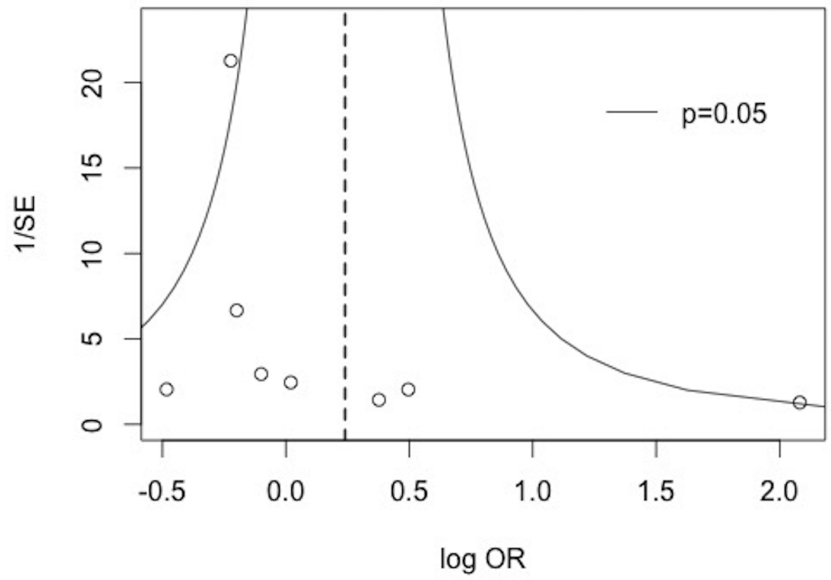


Figure 3.3: Funnel Plot of Hypoxia Studies

The odds ratio and standard error for each of the studies was calculated. The x-axis represents the log of the odds ratio (OR). The y-axis denotes one over the standard error (SE). Un-shaded circles represent individual studies. The vertical dashed central line depicts the mean log odds ratio. The solid vertical curved lines on each side of the dashed lines represent 95% confidence intervals. The plot was generated using R software.

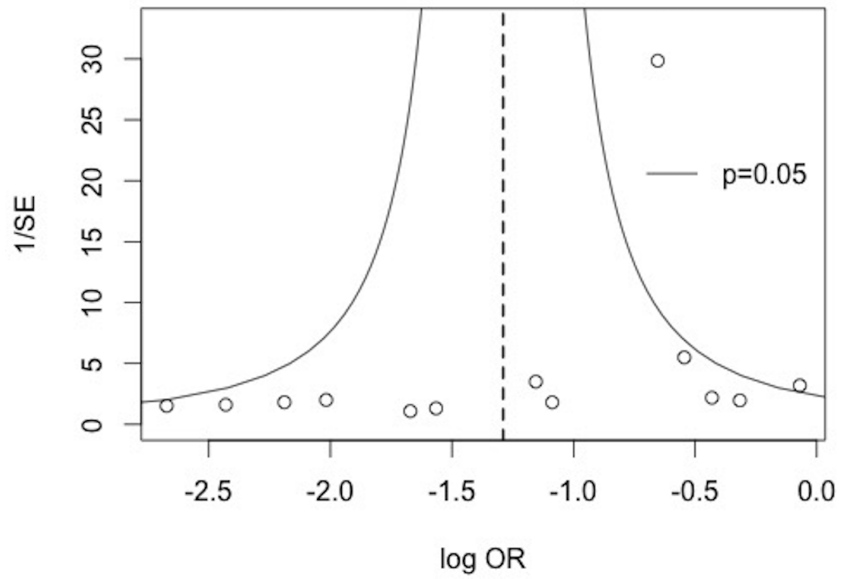


Figure 3.4: Studies Analysed for Bias According to the Newcastle Ottawa Scale. [200]

The studies were scored a maximum four stars for the selection of studies according to predefined criteria, two stars for the comparability of groups and three stars for the studies having the same outcome.

Study	Selection Max = 4 ★	Comparability Max = 2 ★	Outcome Max = 3 ★
Castillo 2012	★ ★ ★ ★		★ ★ ★
Ferguson 2012	★ ★ ★ ★	★ ★	★ ★ ★
Guerra-Wallace 2013	★ ★ ★ ★		★ ★ ★
Puiman 2013	★ ★ ★ ★		★ ★
Bennett 2013	★ ★ ★ ★	★ ★	★ ★ ★
Van Zellem 2015	★ ★ ★ ★	★ ★	★ ★ ★
Smyth 1998	★ ★ ★ ★	★	★ ★
Onyango 1993	★ ★ ★ ★	★	★ ★
Orimadegun 2013	★ ★ ★ ★	★	★ ★
Orimadegun 2014	★ ★ ★	★	★ ★
Chisti 2012	★ ★ ★ ★	★	★ ★
Chisti 2013	★ ★ ★ ★	★ ★	★ ★
Michaud 1992	★ ★ ★ ★		★ ★ ★
Ramaiah 2013	★ ★ ★ ★	★ ★	★ ★ ★
Pigula 1993	★ ★ ★ ★	★ ★	★ ★ ★

Hypoxia

The crude mortality from all the hypoxia studies was 26.2% for the hypoxia group and 19.9% in the normoxia group.

Eleven studies of 5280 children revealed a higher combined odds ratio of mortality

with hypoxia (PaO_2 <60 mmHg (8 kPa) or peripheral oxygen saturations <90%) compared to normoxia (OR: 3.13, 95%CI: 1.79-5.48, p <0.001). The I^2 was 86% indicating a high level of heterogeneity.

The analysis of hypoxia studies (14) with alternative threshold (n=5549) also showed a higher combined odds ratio of mortality with hypoxia (PaO_2 <60 mmHg (8 kPa) or peripheral oxygen saturations <92%) compared to normoxia (OR 2.82, 95%:1.76-4.52). A summary of all published studies is shown in a forest plots. (Figure 3.16)

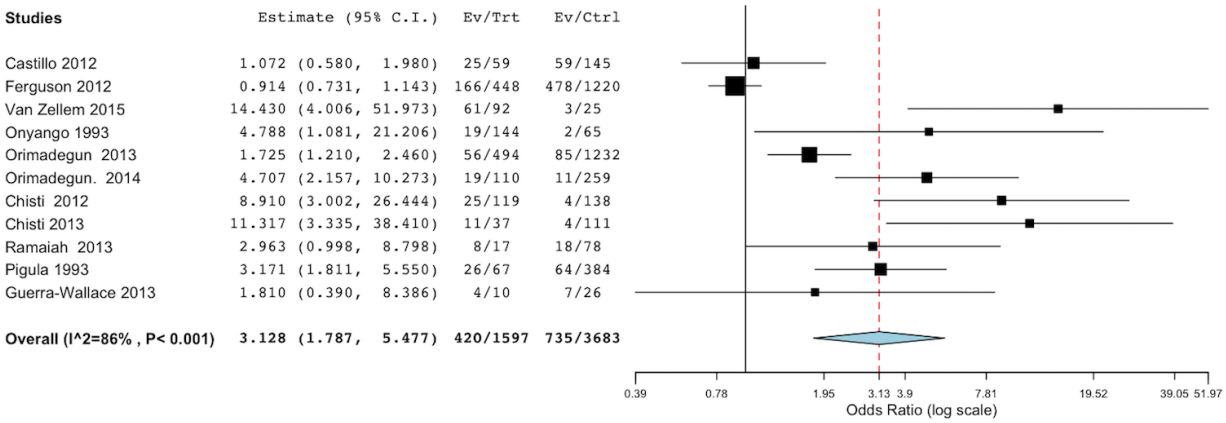
For subgroup analysis, the studies were divided into patients admitted with LRTI or CA or TBI. The single studies in children with malaria and diarrhoea were excluded from the subgroup analysis.

Table 3.1: Hypoxia analysis - Weights of individual studies

Study name	**Weight**
Del Castillo [202]	10.67%
Ferguson [203]	12.00%
Van Zellem [204]	7.46%
Onyango [205]	6.56%
Orimadegun 2013 [206]	11.66%
Orimadegun 2014 [207]	9.88%
Chisti 2012 [208]	8.37%
Chisti 2013 [209]	7.73%
Ramaiah [210]	8.37%
Pigula [211]	10.90%
Guerra-Wallace [212]	6.38%

Figure 3.5: Forest Plot of all Atudies Exploring the Impact of Hypoxia on Mortality

The estimate assessed is the odds ratio of mortality. I^2 is the measure of heterogeneity. The width of the horizontal line for each study represents the 95% confidence interval (CI). The red vertical dashed line represents the overall odds of mortality. The blue rhomboid represents the 95% CI of the overall odds ratio.



Subgroup Analysis of Hypoxia Studies

Children admitted following cardiac arrest

The crude mortality of the CA subgroup was 42.0% in the hypoxia group and 38.6% in the normoxia group. The CA subgroup (3 studies, n=2025) showed a significantly higher mortality with hypoxia compared to normoxia (OR: 2.03, 95%CI: 0.61-6.77, p = 0.352). The measure of heterogeneity (I^2) was 83% (p <0.001).

Children admitted with lower respiratory tract infection

The crude mortality of LRTI subgroup was 12.7% in the hypoxia group and 6.4% in the normoxia group. The subgroup analysis shows higher odds of mortality with

hypoxia compared to normoxia (OR: 3.99, 95%CI: 1.23-12.97, p = 0.021). The I^2 was high (79%, p = 0.008). There were differences in the selection criteria within this subgroup (3 studies, n=2083) that make interpretation of the results difficult.

Children admitted following traumatic brain injury

The crude mortality of the TBI subgroup was 40.5% in the hypoxia group and 17.7% in the normoxia group. The TBI subgroup (2 studies, n=546) displayed higher mortality with hypoxia (OR: 3.12, 95%CI: 1.9-5.14, p <0.001). However, the oxygen thresholds were different between the studies. Despite this I^2 was 0% (p = 0.914) suggesting no heterogeneity. Tables 3.11-3.18 give a summary of the characteristics recorded from the studies including odds ratios and outcome measures.

Table 3.2: Subgroup analysis of hypoxia studies

Subgroup	**Estimate**	**Lower bound**	**Upper bound**	**Std.err**	**p-value**
CA subgroup (4 studies)	2.03	0.61	6.77	0.61	0.25
LRTI subgroup (3 studies)	3.99	1.23	12.96	0.60	0.02
TBI subgroup (2 studies)	3.13	1.78	5.47	0.28	<0.001

Key: CA - Cardiac arrest, LRTI - Lower respiratory tract infection, TBI - Traumatic brain injury

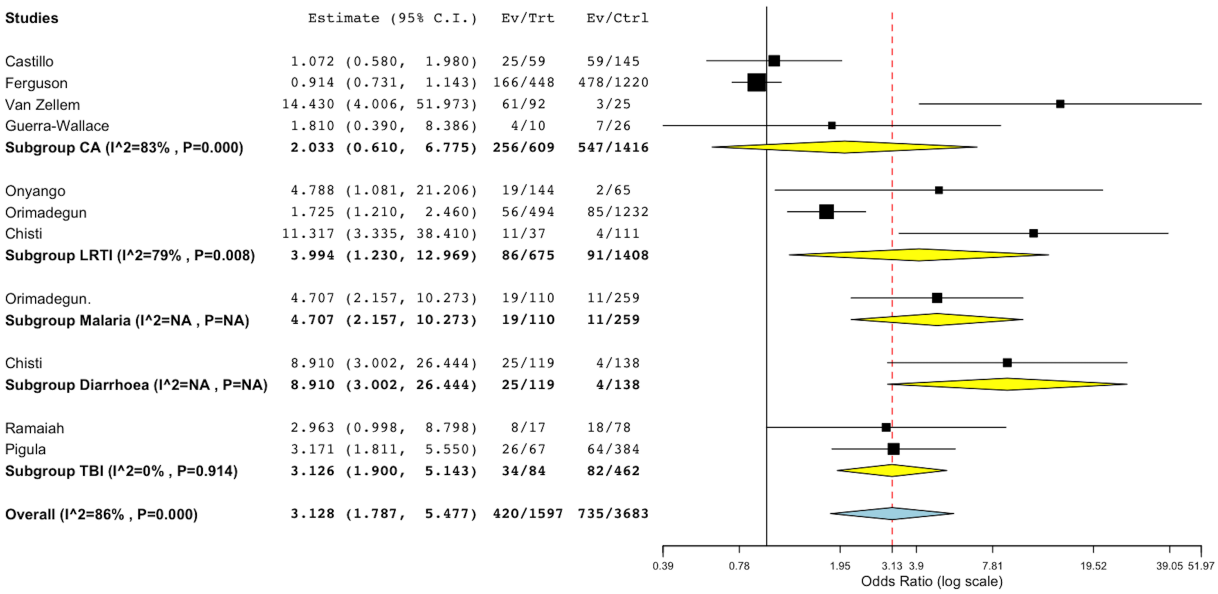
Table 3.3: Heterogeneity analysis of hypoxia studies

Studies	**Het. p-value**	I^2
CA subgroup	<0.001	83%
LRTI subgroup	0.008	79%
TBI subgroup	0.914	0%

Key: CA - Cardiac arrest, LRTI - Lower respiratory tract infection, TBI - Traumatic brain injury

Figure 3.6: Forest Plot Showing Subgroup Analysis of Hypoxia Studies

The estimate assessed is the odds ratio of mortality. I^2 is the measure of heterogeneity. The width of the horizontal line for each study represents the 95% confidence interval (CI). The red vertical dashed line represents the overall odds of mortality. The blue rhomboid represents the 95% CI of the overall odds ratio. The yellow rhomboid represents the 95% CI of the odds ratio of the three subgroups odds. CA - Cardiac arrest subgroup, TBI - Traumatic brain injury subgroup, LRTI - Lower respiratory tract infection subgroup.



Hyperoxia

The crude mortality from all the hyperoxia studies was 37.5% for the hyperoxia group and 38.4% in the normoxia group.

Six studies of 2012 children revealed no effect on mortality. The combined odds

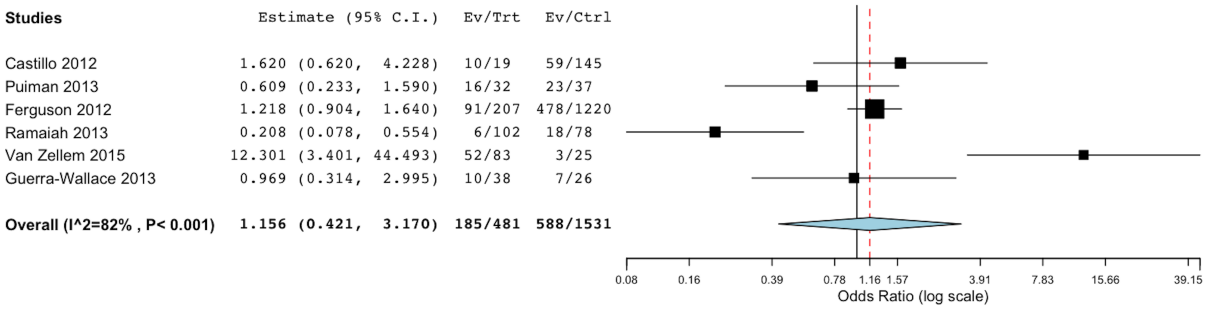
ratio of death with hyperoxia (PaO_2 >300 mmHg (40 kPa)) compared to normoxia was 1.15 (95%CI: 0.42-3.17, p = 0.77). The I^2 was 82% indicating a high level of heterogeneity. For subgroup analysis, the studies were divided into patients admitted following CA or TBI. The analysis of all eight hyperoxia studies (alternative threshold, n= 2244) also showed no effect on mortality (OR 1.31, 95%CI:0.54-2.36, p = 0.74). (Tables 1.4 and Figure 3.18)

Table 3.4: Hyperoxia analysis - Weights of individual studies

Study name	**Weight**
Del Castillo [202]	16.69%
Puiman [213]	16.69%
Ferguson [203]	19.33%
Ramaiah [210]	16.59%
Van Zellem [204]	14.90%
Guerra-Wallace [212]	15.78%

Figure 3.7: Forest Plot of Hyperoxia Studies

The estimate assessed is the odds ratio of mortality. I^2 is the measure of heterogeneity. The width of the horizontal line for each study represents the 95% confidence interval. The red vertical dashed line represents the overall odds of mortality. The blue rhomboid represents the 95% confidence interval of the overall odds ratio.



Subgroup Analysis of Hyperoxia Studies

Children admitted following cardaic arrest

The crude mortality of the CA subgroup was 47.2% in the hyperoxia group and 39.2% in the normoxia group. The CA subgroup (5 studies, n=1832) displayed higher odds of mortality with hyperoxia compared to normoxia (OR: 1.59, 95%CI: 0.64-3.91, p = 0.313). The studies were retrospective except the Del Castillos prospective multicenter study.(19) The measure of heterogeneity (I^2) was 73% (p = 0.005).

The leave-out-one-study analysis showed that the effect estimate (odds ratio of

mortality) was unaffected by the variation in outcome measure between studies.

Children admitted following traumatic brain injury

No subgroup analysis was possible since only a single study investigated children with TBI. With an alternative threshold, 2 studies were included. The crude mortality of the TBI subgroup was 12.1% in the hyperoxia group and 24.4% in the normoxia group. The TBI subgroup (2 studies, n=239) revealed lower mortality with hyperoxia compared to normoxia (OR: 0.34, 95%CI: 0.10-1.08, p = 0.06). The heterogeneity measure was 48% (p=0.16).

Table 3.5: Subgroup analysis of hyperoxia studies

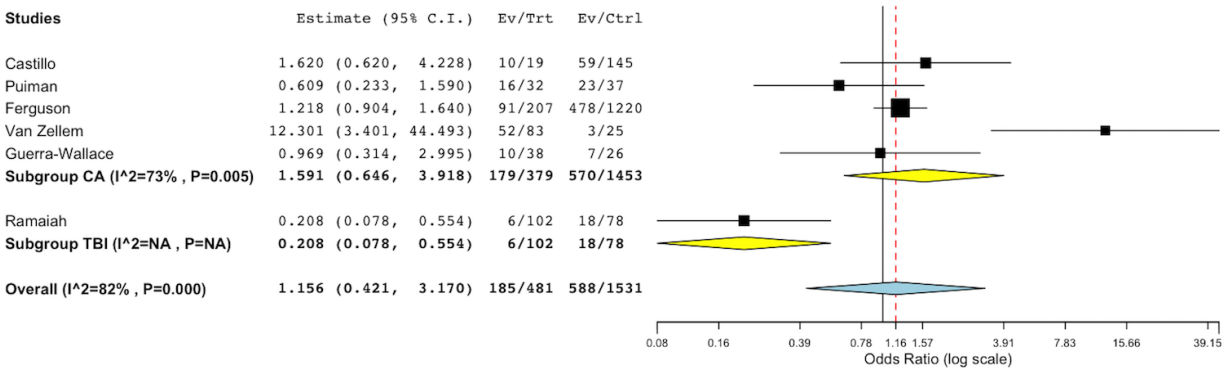
Subgroup	**Estimate**	**Lower bound**	**Upper bound**	**Std.err**	**p-value**
Cardiac arrest (5 studies)	1.59	0.64	3.91	0.46	0.31

Table 3.6: Heterogeneity analysis of hyperoxia studies

Studies	**Het. p-value**	I^2
Cardiac arrest subgroup	0.005	73%

Figure 3.8: Forest Plot for Subgroup Analysis of Hyperoxia Studies

The estimate assessed is the odds ratio of mortality. I^2 is the measure of heterogeneity. The width of the horizontal line for each study represents the 95% confidence interval (CI). The red vertical dashed line represents the overall odds of mortality. The blue rhomboid represents the 95% CI of the overall odds ratio. The yellow rhomboid represents the 95% CI of the odds ratio of the two subgroups odds. CA cardiac arrest subgroup, TBI Traumatic brain injury subgroup.



Results of the Meta-regression

Further analysis was performed using meta-regression using the Newcastle Ottawa Scale as a moderator. The beta-coefficient for the Newcastle Ottawa Scale was -0.106 (95%CI: - 0.367-0.154, p = 0.424). (Figure 3.15)

Figure 3.9: Meta-Regression analysis of hyperoxia studies using the Newcastle Ottawa Scale as a moderator

This analysis included the Newcastle Ottawa Scale (NOS) as the independent variable and log odds ratio of each of the studies as the outcome variable. The odds ratio represents the odds of death with hyperoxia compared to normoxia. The diameter of the circles are proportional to the sample size. There were 8 studies included in this analysis. The blue line is the regression line denoting the relationship between NOS and log odds ratio.

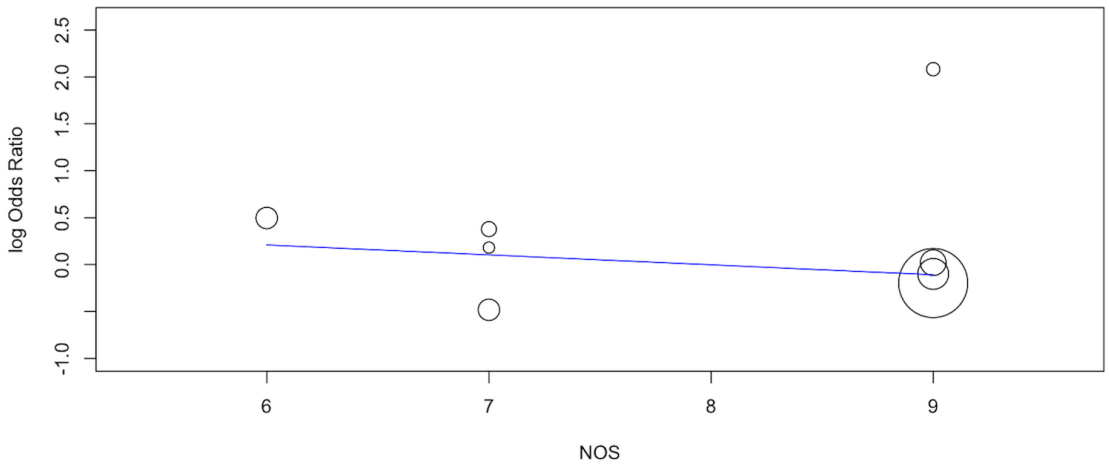


Table 3.7: Characteristics of hyperoxia studies - 1

Author	**Study design**	**Hyperoxia threshold**	**Hypoxia threshold**
Post cardiac arrest			
Del Castillo [202]	Prospective cohort study	>300 mmHg (40 kPa)	<60 mmHg (8 kPa)
Ferguson [203]	Retrospective observational study	>600 mmHg (40 kPa)	<60 mmHg (8 kPa)
Guerra-Wallace [212]	Retrospective cohort study	>300 mmHg (40 kPa)	<60 mmHg (8 kPa)
Puiman [213]	Retrospective cohort study	>300 mmHg (40 kPa)	None
Bennett [214]	Retrospective cohort study	>200 mmHg (26.7kPa)	<50 mmHg (4 kPa)
Van Zellem [204]	Retrospective cohort study	> 300 mmHg (40 kPa)	<60 mmHg (8 kPa)
Traumatic brain injury			
Michaud [215]	Retrospective cohort study	>350 mmHg (46 kPa)	<105 mmHg (13.8 kPa)
Ramaiah [210]	Retrospective cohort study	>300 mmHg (40 kPa)	<60 mmHg (8 kPa)

Legend: The control population included normoxic children for all the studies.

Table 3.8: Characteristics of hyperoxia studies - 2

Author	**Age**	**Sample size**	**Outcome**
Post cardiac arrest			
Del Castillo [202]	1 month to 18 years	223	In-hospital mortality
Ferguson [203]	<16 years	1875	In-PICU mortality
Guerra-Wallace [212]	<18 years	74	Mortality at 6 months
Puiman [213]	Unclear	67	In-hospital mortality
Bennett [214]	>24 hrs to 18 years	195	In-hospital mortality
Van Zellem [204]	>28 days to 18 years	200	In-hospital mortality
Traumatic brain injury			
Michaud [215]	<16 years	75	In-hospital mortality
Ramaiah [210]	<14 years	194	In-hospital mortality

Key: PICU - Paediatric intensive care unit

Table 3.9: Characteristics of hyperoxia studies - Actual numbers

Author	**Hyperoxia**		**Normoxia**		**Hypoxia**	
	Total exposed	Survived	Total exposed	Survived	Total exposed	Survived
Post cardiac arrest						
Del Castillo [202]	19	9	145	86	59	34
Ferguson [203]	207	115	1220	743	448	282
Guerra-Wallace [212]	38		26		10	
Puiman [213]	32	16	37	14		
Bennett [214]	107	36	66	29	22	8
Van Zellem [204]	83	31	25	22	92	31
Traumatic brain injury						
Michaud [215]	47	35	12	8	11	3
Ramaiah [210]	99	93	78	60	17	9

Table 3.10: Characteristics of hyperoxia studies - Odds ratios

Author	**Unadjusted OR (95%CI)**	**Adjusted OR (95%CI)**	**Co-variates**
Post cardiac arrest			
Del Castillo [202]	0.61 (0.24-1.61)	N/A	N/A
Ferguson [203]	N/A	0.8 (0.73-0.87)	Age, sex, ethnicity, CHD, OOHCA, year, PIM2, organ support
Guerra-Wallace [212]	N/A	1.19 (0.19-7.38)	PaO_2 <60 mmHg and PaO_2 >200 mmHg
Puiman [213]	1.64 (0.63-4.29)	N/A	N/A
Bennett [214]	N/A	1.02 (0.46-2.27)	Age, arrest location, arrest rhythm, epinephrine doses
Van Zellem [204]	1.38 (0.782.47)	0.905 (0.46-1.76)	Age, gender, BLS or APLS, rhythm, lowest pH and highest lactate
Traumatic brain injury			
Michaud [215]	1.46 (0.37-5.72)	N/A	N/A
Ramaiah [210]	N/A	8.02 (1.73-37.18)	Older age, lower injury severity score, $paCO_2$ 36-45 mmHg

Key: OR - Odds ratio, CI - Confidence interval, PaO_2 - Arterial oxygen tension, $PaCO_2$ - Arterial carbon dioxide tension, BLS - Basic life support, APLS - Advanced paediatric life support, CHD - Congenital heart disease, OOHCA - out of hospital cardiac arrest, PIM2 - Paediatric index of mortality 2,
N/A - Not applicable

Table 3.11: Characteristics of hypoxia studies - 1

Author	**Study design**	**Hypoxia threshold**	**Control**	**Age**
Smyth [216]	Prospective observational cohort	<92%	≥92%	<5 yrs
Onyango [205]	Prospective observational cohort	<90%	≥90%	<3 yrs
Orimadegun (2013) [206]	Prospective cohort	≤90%	>90%	<15 yrs
Orimadegun (2014) [207]	Prospective cohort	<90%	≥90%	<5 yrs
Chisti (2012) [208]	Prospective cohort	<90%	≥90%	<5 yrs
Chisti (2013) [209]	Prospective case-control (unmatched)	<90%	≥90%	<5 yrs
Pigula [211]	Prospective cohort	PaO_2 <60 mmHg	>60 mmHg	<17 yrs

Key: PaO_2 - arterial partial pressure of oxygen

Table 3.12: Characteristics of hypoxia studies - 2

Author	**Age**	**Sample size**	**Outcome**
Smyth [216]	LRTI	158	Survival to hospital discharge
Onyango [205]	LRTI	256	Survival on day 5
Orimadegun (2013) [206]	LRTI	1726	Survival to hospital discharge
Orimadegun (2014) [207]	Malaria	369	Survival to hospital discharge
Chisti (2012) [208]	Diarrhoea	258	Survival to hospital discharge
Chisti (2013) [209]	LRTI	148	Survival to hospital discharge
Pigula [211]	TBI	451	Survival to hospital discharge

Key: LRTI - Lower respiratory tract infection, TBI Traumatic brain injury, PaO_2 - arterial oxygen tension

Table 3.13: Characteristics of hypoxia studies - Actual numbers

Author	**Normoxia**	**Normoxia**	**Hypoxia**	**Hypoxia**
	Total exposed	Survived	Total exposed	Survived
Post cardiac arrest				
Del Castillo [202]	145	86	59	34
Ferguson [203]				
Bennett [214]	66	29	22	8
Van Zellem [204]	25	22	92	31
LRTI, Diarrhoea or Malaria				
Smyth [216]	103	90	55	45
Onyango [205]	65	63	144	125
Orimadegun (2013) [206]	1232	1147	494	438
Orimadegun (2014) [207]				
Chisti (2012) [208]	138	134	119	94
Chisti (2013) [209]	111	107	37	26
Traumatic brain injury				
Michaud [215]	12	8	11	3
Ramaiah [210]	78	60	17	9
Pigula [211]	384	320	67	41

Table 3.14: Characteristics of hypoxia studies - Odds ratios

Author	**Unadjusted OR (95%CI)**	**Adjusted OR (95%CI)**	**Co-variates**
Post cardiac arrest			
Del Castillo [202]	0.933 (0.505-1.723)	N/A	N/A
Ferguson [203]	0.52 (0.487-0.555)	N/A	N/A
Bennett [214]	0.729 (0.269-1.973)	N/A	N/A
Van Zellem [204]	0.069 (0.019-0.25)	N/A	N/A
LRTI, Diarrhoea or Malaria			
Smyth [216]	0.65 (0.265-1.597)	N/A	N/A
Onyango [205]	0.209 (0.047-0.925)	N/A	N/A
Orimadegun (2013) [206]	0.58 (0.406-0.827)	N/A	N/A
Orimadegun (2014) [207]	N/A	0.133 (0.05-0.357)	Metabolic acidosis,hypoxaemia, hypoglycemia
Chisti (2012) [208]	0.112 (0.038-0.333)	N/A	N/A
Chisti (2013) [209]	0.088 (0.026-0.3)	N/A	N/A
Traumatic brain injury			
Michaud [215]	0.188 (0.031-1.122)	N/A	N/A
Ramaiah [210]	0.337 (0.114-1.002)	N/A	N/A
Pigula [211]	0.315 (0.180-0.552)	N/A	N/A

Key: OR - Odds ratio, CI Confidence interval, N/A Not applicable, LRTI - Lower respiratory tract infection

In summary, the systematic review shows an increased odds of mortality with hypoxia (OR-3.13). The evidence from hyperoxia is unclear. I will now detail the observational studies followed by the national survey.

3.3 Observational Studies

3.3.1 Methods

3.3.2 Extreme Hypoxia Threshold Study

The null hypothesis of this study was that 'extreme hypoxaemia' is not survivable. 'Extreme hypoxaemia' was defined as an admission arterial partial pressure of oxygen (PaO_2) <2.67 kPa (20 mmHg). This cut off was chosen as per the Gray paper. Historically patients with values below this threshold were expected to die. However the Gray study from 1970 showed that some patients survived to hospital discharge with values below this limit. [9] More recently, Grocott observed a PaO_2 of 19.1 mmHg in a healthy adult mountaineer. [10] Therefore, I aimed to disprove the null hypothesis and explore the theory that adaptative mechanisms might be active even during acute hypoxia.

This retrospective observational study was performed in GOSH. All admissions to critical care services over an 11-year period (Jan 2004 - December 2014) were

included for analysis. Repeat admissions were excluded. The incidence of 'extreme hypoxaemia' was explored.

3.3.3 PaO_2 - Mortality Continuous Variable Study

For this part of the observational study PaO_2 was employed as a continuous variable. The relationship between admission arterial PaO_2 ranging from 1 to the maximum recorded value of 147 kPa and mortality at intensive care unit discharge was investigated. All admissions to critical care services over 11-years (Jan 2004 - December 2014) were utilised for this analysis. Children with no documented admission PaO_2 or FiO_2 were excluded. Further analysis was performed with thresholds. Hypoxia was defined as PaO_2 <8 kPa, normoxia as PaO_2 between 8.1-40 kPa and hyperoxia as PaO_2 >40 kPa. These cut-offs were selected in accordance with the paper by Kilgannon. [11]

3.4 Statistical Analysis

3.4.1 Extreme Hypoxia Threshold Study

The incidence of 'extreme hypoxia' was presented as absolute numbers with the denominator as total admissions to the critical care services over the study period with a full dataset. Children with a cardiac pathology were analysed separately

since some of them may have been hypoxic for a longer period (cyanotic conditions). I wished to investigate if being hypoxic for a longer duration conferred a beneficial effect on eventual survival. Therefore within the 'extreme hypoxaemia' group, Fishers exact test of significance was used to compare survivors with and without cardiac aetiology.

3.4.2 PaO_2 - Mortality Continuous Variable Study

A logistic regression model was employed to assess the admission arterial PaO_2 and mortality relationship. Following this, a regression curve estimation technique in SPSS (IBM software, V21.0) was utilised to explore the relationship between admission PaO_2 and probability of death. The most parsimonious model was chosen as the final best fit using goodness of fit method (Bayesian Information Criterion). A model is considered more parsimonious if it is of a lower complexity. For example, the hierarchy might be linear, logarithmic, quadratic, cubic, logistic and so on. This was the quadratic function in my case. The equation was as follows:

Predicted mortality = Constant + (b1 * PaO_2) + (b2 * $(PaO_2)^2$)

Patients were empirically categorised into 6 groups according to their admission

PaO_2 (<8, 8.1-10, 10.1-13, 13.1-25, 25.1-40 and >40 kPa).

The Paediatric Index of Mortality 2 (PIM2) is a risk prediction score. It enables the comparison of care delivered by intensive care units by creating a score from specifically chosen admission variables. Admission PaO_2 is one of the variables included in the model. [217] In order to delineate the effect of PaO_2 on the outcome, the logit equation for the Paediatric index of mortality 2 (PIM2) was amended. The coefficient of FiO_2/PaO_2 was removed from the PIM2 equation to create a modified PIM2 (mPIM2). Non-linear regression analysis was employed to analyse the effect of co-variates (admission PaO_2, age, ethnicity and mPIM2) on mortality. In addition, the PIM2 and Paediatric Index of Mortality 3 (PIM3) risk of mortality vs PaO_2 curves were analysed. The aim was to analyse the contribution and impact of PaO_2 to the prediction model. All variables in the model were kept constant (using the values quoted for 'model patient' described in the PIM3 article). [218] Then when PaO_2 was varied the curve represented univariate relationship to the predicted mortality.

The Standardised Mortality Ratio (SMR) was calculated as per the equation:

SMR = Observed deaths / Expected deaths

The expected deaths were calculated from the PIM2 value. A modified SMR

(mSMR) was determined by using the mPIM2 in place of PIM2. The relationship between mSMR, SMR and admission PaO_2 was investigated. Standardised mortality ratio, mSMR and 95% confidence intervals were plotted for each of the pre-defined groups.

3.5 Results

3.5.1 Extreme Hypoxia Threshold Study

A total of 14,308 children were admitted to the critical care services during the study period. On average, there were 1635 admissions per year to the critical care services at GOSH over the study period. Complete datasets were available for 7751 patients.

The admission PaO_2 was <20 mmHg in 40 children (0.5%). The median weight in this group was 3.21 kgs (LCI 3.1, UCI 4.02). Twenty seven of them were admitted to the cardiac intensive care unit, 7 to the neonatal intensive care unit and 6 to the paediatric intensive care unit. The etiology was a cardiac pathology in 19 children. The rest were: 13 respiratory, 2 neurological, 1 general surgical post-op and 5 miscellaneous disorders. Nearly 57% (23) patients had previous ICU admissions.

Of the 40 children, 33 (82.5%) were alive at intensive care unit discharge. There

were 3382 (43.6%) patients with a cardiac aetiology. Children with and without a cardiac aetiology did not have a statistically significant difference in mortality (Fishers exact p = 0.68). Table 3.1 shows the number of children according to cardiac aetiology and survival.

Table 3.15: Extreme hypoxaemia: Survival to intensive care unit discharge vs. Aetiology

Aetiology	**Cardiac disease**	**Others**	**Total**
Survival to ICU discharge			
Yes	15	18	33
No	4	3	7

Legend: Extreme hypoxia was defined as admission PaO_2 of <20 mmHg. The total number of children with 'extreme hypoxia' was 40. The table displays the outcome of children separated as either having a cardiac aetiology or other. The outcome is similar between the groups (Fishers exact - p = 0.68). ICU - Intensive care unit.

3.5.2 PaO_2 - Mortality Continuous Variable Study

Data from all admissions to critical care services in Great Ormond Street Hospital over an 11-year time interval was reviewed. Of 14,321 total admissions during the

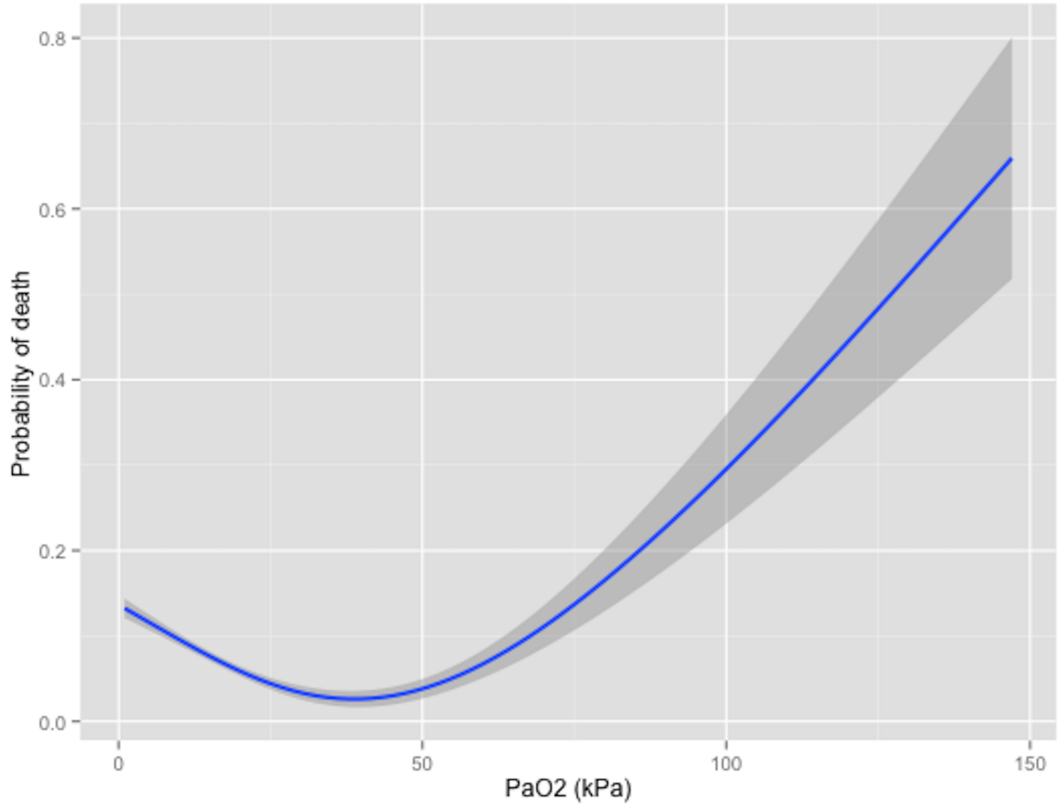
study period, 7410 patients had a documented admission PaO_2 in the first hour.

In all, 1324 (17.8%) of these patients were hypoxic, 5385 (72.6%) were normoxic and 701 (9.4%) were hyperoxic on admission. The crude mortality was 204 (15.4%) in the hypoxic group, 287 (5.3%) in the normoxic group and 64 (9.1%) in the hyperoxic group. PaO_2 and crude mortality was found to have a quadratic (U-shaped) relationship.(Figure 3.1) The table shows the coefficients for non-linear regression analysis with PaO_2, age, gender and mPIM as co-variates.(Table 3.2) On the basis of the primary diagnosis on admission, the patients with cyanotic congenital heart disease were identified and separately analysed. The U-shaped relationship between PaO_2 and crude mortality was preserved even in this subgroup of children with cyanotic cardiac disorders.

The 'U-shaped' relationship suggests that probability of death increases both at low and high PaO_2 level.

Figure 3.10: Relationship between admission PaO_2 (kPa) and probability of death

Relationship between arterial partial pressure of oxygen on admission and unadjusted mortality in 7410 critically ill children admitted to Great Ormond St Hospital Intensive Care 2004-2014. The regression curve estimation shows that PaO_2 mortality relationship is a quadratic function (U-shaped curve).



The PIM2 and PIM3 mortality risk prediction score had an inverse power law relationship to admission PaO_2. This relationship was consistent across all risk categories of patients. (Figures 3.2 and 3.3)

The equation for PIM2 is:

PIM2 = (0.016*absolute (systolic blood pressure)-120)) + (4.0767 * pupillary reaction) + (0.2074*100*(FiO2/PaO2)) + (0.0671* absolute base excess)) + (0.6999*(mechanical ventilation)) - (0.6496*(elective admission)) - (1.1876*(surgical recovery))+ (0.0866*(cardiac bypass)) + (1.408*(high risk diagnosis)) - (1.8624*(low risk diagnosis)) - 4.4585

The equation for PIM3 is:

PIM3 = (3.8233*pupillary reaction) + (0.5378*elective admission) + (0.9763*mechanical ventilation) + (0.0671*absolute base excess) + (0.0431*systolic blood pressure) + (0.1716*(systolic blood pressure /1000) + (0.4214*(FiO2/PaO2)*100) + (1.2246*cardiac bypass) + (0.8762* surgical recovery - non-bypass) + (1.5164* surgical recovery - non-cardiac) + (1.6225*very high risk diagnosis) + (1.0725*high risk diagnosis) + (2.1766 * low risk diagnosis) 1.7928

Figure 3.11: Relationship between admission PaO_2 (kPa) and PIM2 predicted risk of mortality

Relationship between arterial partial pressure of oxygen on admission and risk of mortality as predicted by the PIM2 score. The clinical variables of the 'model patient' described in the PIM3 article were inputted into the PIM2 equation with varying PaO_2. An inverse power law relationship was noted. [218]

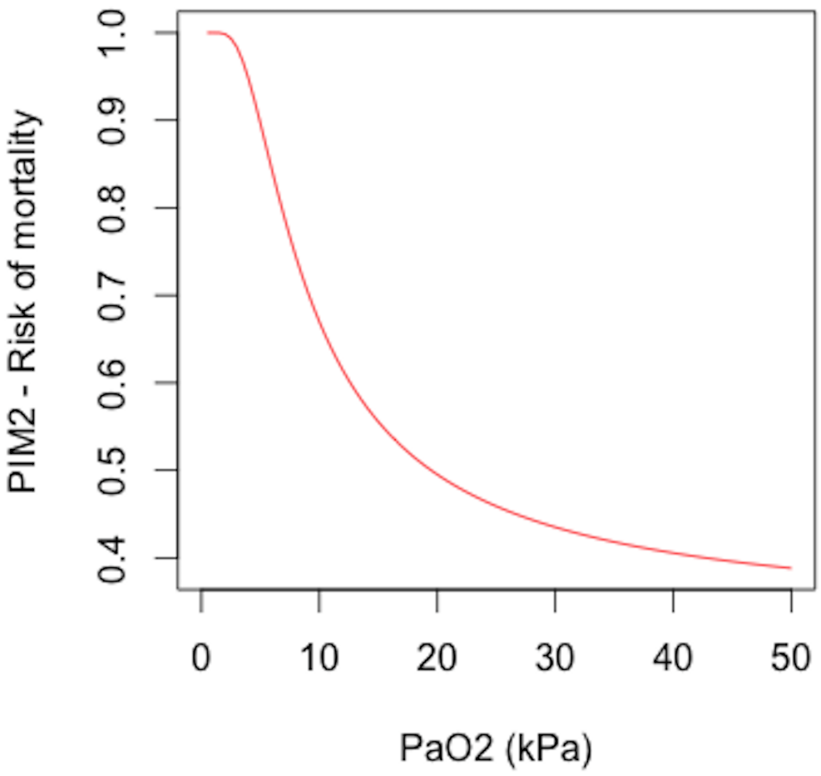
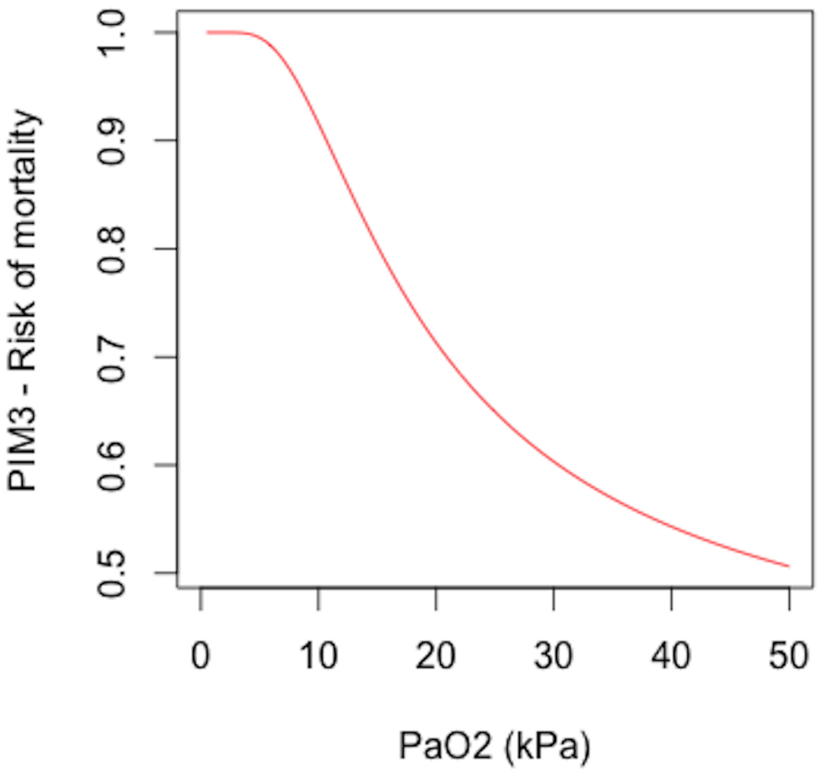


Figure 3.12: Relationship between admission PaO_2 (kPa) and PIM3 predicted risk of mortality

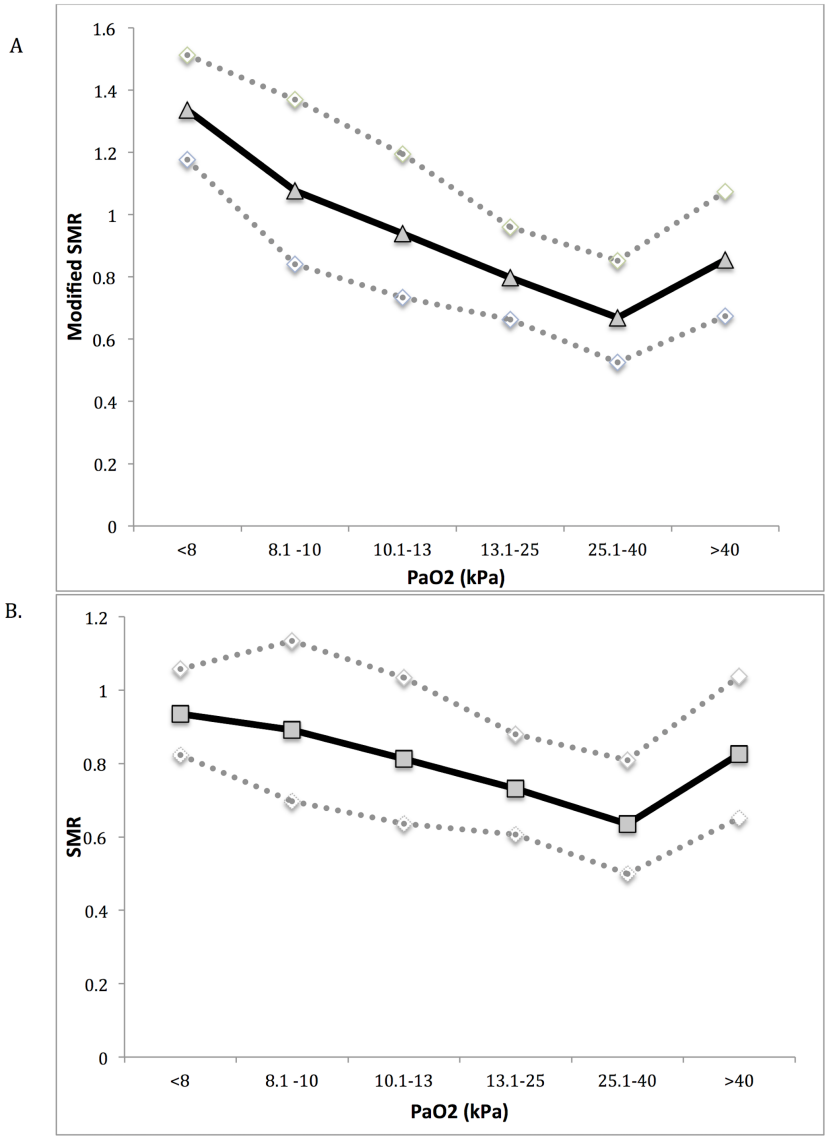
Relationship between arterial partial pressure of oxygen on admission and risk of mortality as predicted by the PIM3 score. The clinical variables of the 'model patient' described in the PIM3 article were inputted into the PIM3 equation with varying PaO_2. A inverse power law relationship was noted. [218]



The standardized mortality ratio (SMR) and modified standardized mortality ratio (mSMR) were 0.93 and 1.34 respectively for the hypoxic group (PaO_2 <8 kPa), 0.75 and 0.83 for the normoxic group (8-40 kPa) and 0.82 and 0.85 for the hyperoxic group (>40 kPa).The mSMR was higher compared to SMR when PaO_2 was less than 13 kPa. This relationship persisted even when the patients were separated into subgroups of cardiac and non-cardiac.

Figure 3.13: Relationship between admission PaO_2 (kPa) and the standardized mortality ratio / modified standardized mortality ratio in all patients

Panel A and B. The relationship between the partial pressure of oxygen (PaO_2) and the modified standardized mortality ratio and standardized mortality ratio. The modified standardised mortality ratio was calculated by excluding the FiO_2 / PaO_2 coefficient from the PIM2 calculation. The standardised mortality ratio was calculated using the paediatric index of mortality 2 (PIM2) score. The grey dashed lines represent 95% upper and lower confidence intervals.



The non-linear regression analysis shows that PaO_2 has a statistically significant association to the outcome (death) in the overall group and less so in the cyanotic cardiac disease subgroup. Of note, the coefficients for PaO_2 are small. The modified SMR, unsurprisingly, also has a statistically significant effect on the outcome. Table 3.2 and table 3.3 show the non-linear regression coefficients.

Table 3.16: Nonlinear regression - Death as outcome - All admissions

Co-variates	**Estimate**	**Std.err**	**p-value**
$(PaO_2)^2$	3.99×10^{-5}	7.38×10^{-6}	<0.0001
PaO_2	-3.63×10^{-3}	5.26×10^{-4}	<0.0001
Age	6.23×10^{-5}	4.96×10^{-5}	0.21
Sex	-9.11×10^{-3}	5.60×10^{-3}	0.10
Modified PIM2	0.82	0.02	<0.0001

Legend: Coefficients of non-linear regression model. The model includes all patients with valid data. Death is the outcome. Age and sex do not have a statistically significant effect on the outcome. Modified PIM2 has the largest effect on the outcome. PaO_2 has a small but statistically significant effect on the outcome.

Table 3.17: Nonlinear regression - Death as outcome - Cyanotic cardiac disease subgroup

Co-variates	**Estimate**	**Std.err**	**p-value**
$(PaO_2)^2$	4.53×10^{-5}	2.39×10^{-5}	0.06
PaO_2	-2.91×10^{-3}	1.09×10^{-3}	0.007
Age	-5.17×10^{-5}	7.47×10^{-5}	0.488
Sex	-0.013	0.007	0.073
Modified PIM2	0.52	0.05	<0.0001

Legend: Coefficients of non-linear regression model. The model includes patients with cyanotic cardiac disease with valid data. Death is the outcome. Age, sex and PaO_2 do not have a statistically significant effect on the outcome. Modified PIM2 has the largest effect on the outcome. PaO_2 has a small but statistically significant effect on the outcome.

3.6 Survey

The survey was designed to describe the UK clinical practice in respect of supplemental oxygen therapy in ventilated critically ill children. Similar surveys have proved to be valid tools for describing true variability in clinical practice and feasibility steps to randomized clinical trials. [219] [220] A web-based tool was

employed to facilitate data collection using a University College London website (opino.ucl.ac.uk.).

All members of the Paediatric Intensive Care Society (PICS), UK, were requested by email to complete the survey via a weblink that would only permit a single contribution from each email. Paediatric Intensive Care Society membership consists of approximately 800 nursing, medical and allied health professionals working in paediatric intensive care units. Clinical staff from neonatal intensive care units in the UK were not approached, as their patient profile was considered to be significantly different.

Demographic data, including age, intensive care unit type, their seniority and years of practice were sought. After discussion of this study with the chair of Bloomsbury Research and Ethics Committee (London, UK), I was advised that a formal ethics review was not required.

The survey outlined the following clinical scenario:

A 1-year-old with no premorbid conditions is ventilated with peak inspiratory pressure of 28 cm H_2O, positive end expiratory pressure of 6 cm H_2O, respiratory rate of 20 breaths per min and FiO_2 of 0.8. His peripheral oxygen saturation (pulse oximetry), heart rate, blood pressure and mean blood pressure are 94%, 125 beats per min, 85/56 mmHg and 66 mmHg respectively. He has bilaterally equal and

reactive pupils measuring 3 mm. He is sedated on intravenous morphine and midazolam. He is not paralysed. Latest arterial blood gas values are - pH: 7.32, PCO_2: 6.2 kPa, PaO_2: 10 kPa, BE: -ve 4 and Lactate: 1.5 mmol/L. The PIM2 predicted risk of mortality is 8.8 %. He has been ventilated for 2 days.

With the same clinical history, clinicians were asked to decide on the oxygenation targets when the potential diagnosis is acute respiratory distress syndrome, post-cardiac arrest, sepsis, traumatic brain injury (TBI) or pulmonary hypertension.
A further question examined weaning protocols in their units. Interest in a potential randomised control trial with oxygenation targets was explored (Appendix 1).

3.6.1 Statistical Analysis

Survey results are presented in tables and figures. The characteristics of the respondents are presented as absolute numbers with percentages. The responses to scenarios are reported as histograms.

3.6.2 Results

Only 53 of those who were invited to participate in the online survey responded. The total number invited to participate was unclear as the PICS membership was not well defined. This number was suspected to be about 400. Hence, the response rate was 13%. The majority of respondents worked in moderate sized ICUs, with admission rates between 500-1000 patients per annum. The characteristics of respondents are presented in table 3.4.

Table 3.18: Demographic characteristics of respondents

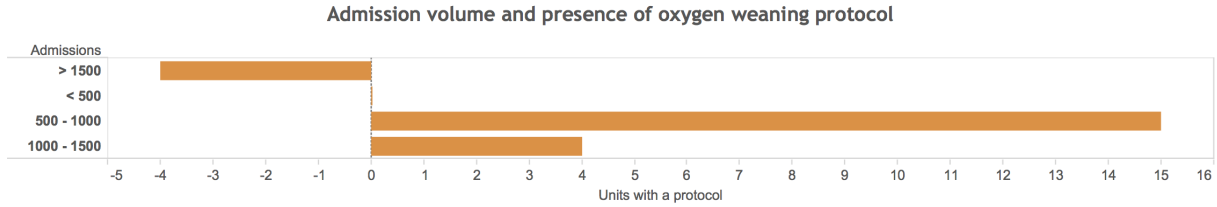
The table gives number of respondents (percentage within brackets) and their characteristics.

Number of admissions to intensive care unit	**Number(%)**
<500	9 (17)
501 1000	24 (47)
1001 1500	13 (25)
>1500	5 (10)
No response	2
Cardio-surgical center	33 (66)
Neuro-surgical center	35 (67)
Age	
20 - 30 years	1 (2)
31 - 40 years	24 (46)
41 - 50 years	19 (36)
>50 years	8 (15)
No response	1
Grade of person filling survey	
Consultant	25 (48)
Senior nurse	10 (19)
Senior fellow	12 (23)
Junior fellow	5 (10)
No response	1
Number of years of practice in intensive care	
2 - 5 years	13 (25)
5 - 6 years	13 (25)
>10 years	25 (49)

The majority of responders (96%) reported having an alarm target on the oxygen saturation monitors in their unit. Thirty-eight respondents (73%) worked in units that did not have an oxygen weaning protocol for mechanically ventilated patients. The units with more than 1500 admissions were less likely to have a weaning protocol compared to those with between 500 and 1500 admissions.(Figure 3.5)

Figure 3.14: Relationship between Volume of Admissions and Presence of an Oxygen Weaning Protocol

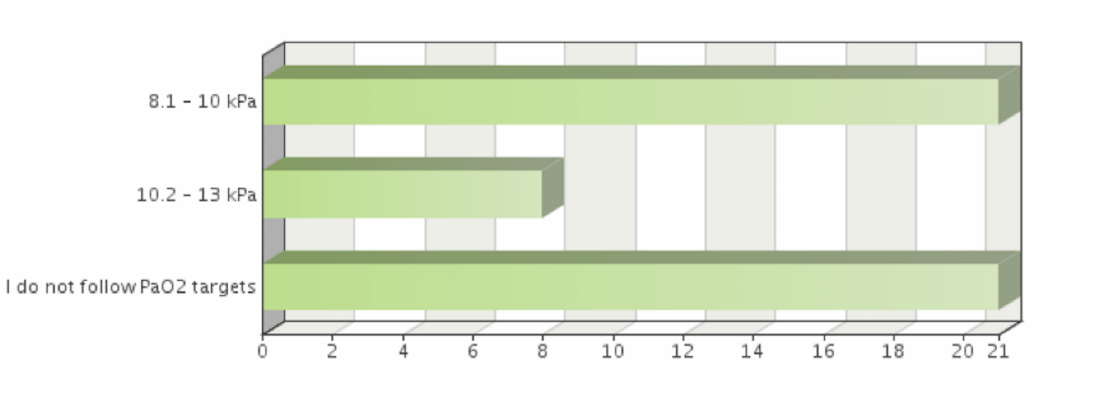
The result was normalised for the units with an admission volume of fewer than 500 cases a year. The largest units were more likely not to have a weaning protocol.



For the given clinical scenario, 21 respondents (42%) did not follow PaO_2 targets. Of the reminder, 21 clinicians (42%) targeted between 8.1 and 10 kPa. Only 8 (16%) aimed for the normal range (10.1-13 kPa).(Figure 3.6)

Figure 3.15: Target of PaO_2

Respondents were classified into three groups according to their PaO_2 target practice. The respondents were requested to choose the range of PaO_2 they would target for the patient with the illustrated clinical scenario. Nearly half the respondents do not follow PaO_2 targets. Of the rest, the majority claim to target slightly lower targets than the normal range.



In acute respiratory distress syndrome, cardiac arrest (CA) and sepsis, there was a tendency to aim for lower PaO_2 (<10 kPa) as the FiO_2 increased. This was noticeable when the FiO_2 was more than 0.4 (45%) which equates to a PaO_2 / FiO_2 ratio of less than 200. Following TBI and in pulmonary hypertension, there was a propensity to aim for normal PaO_2 (10.1-13 kPa) even as the FiO_2 rose (28-33% when FiO_2 >0.4). In TBI, the proportion of respondents targeting a lower PaO_2 increased when the FiO_2 was more than 0.8 (8%). A proportion of respondents targeted PaO_2 ranges above normal (15%). In pulmonary hypertension, normal range remained the preferred choice throughout the range of FiO_2.(Figure 3.7)

Figure 3.16: Target PaO_2 and FiO_2 Profile in Various Clinical Scenarios

The profile of PaO_2 (y-axis) and FiO_2 (x-axis) targeted in 5 clinical scenarios in a child with moderate ventilatory requirements. The PaO_2 ranged from <8 kPa in the top panel to >13 kPa in the bottom panel within each scenario. FiO_2 ranged from 0.21 (blue) through to 0.81-1 (purple). The three scenarios in the top panel show a pattern of more restrictive PaO_2 targets with increasing FiO_2. The 2 scenarios in the lower panel show that higher normal PaO_2 ranges are targeted irrespective of increasing FiO_2.

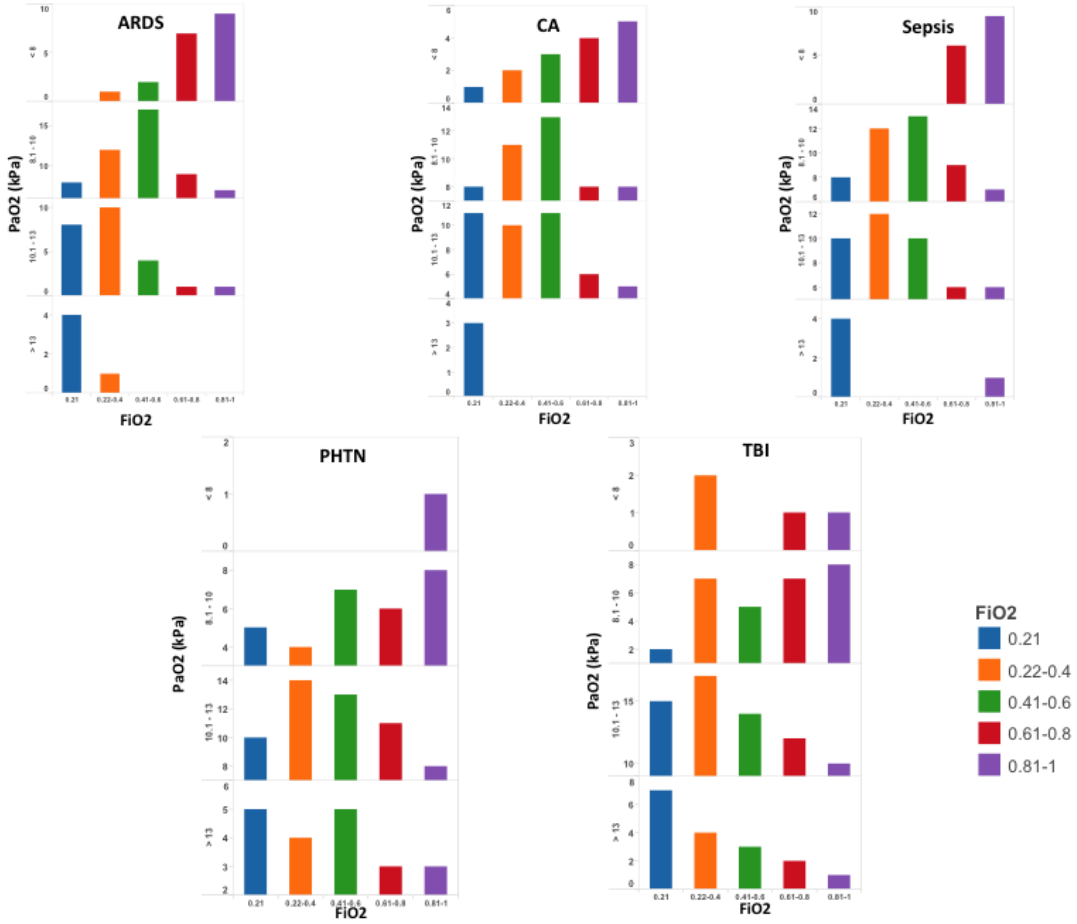


Figure 3.17: Target PaO_2 and FiO_2 Profile in Acute Respiratory Distress Syndrome

The profile of PaO_2 (y-axis) and FiO_2 (x-axis) targeted in a child with ARDS and moderate ventilatory requirements. The PaO_2 ranged from <8 kPa in the top panel to >13 kPa in the bottom panel. FiO_2 ranged from 0.21 (blue) through to 0.81-1 (purple). The pattern is of more restrictive PaO_2 targets with increasing FiO_2.

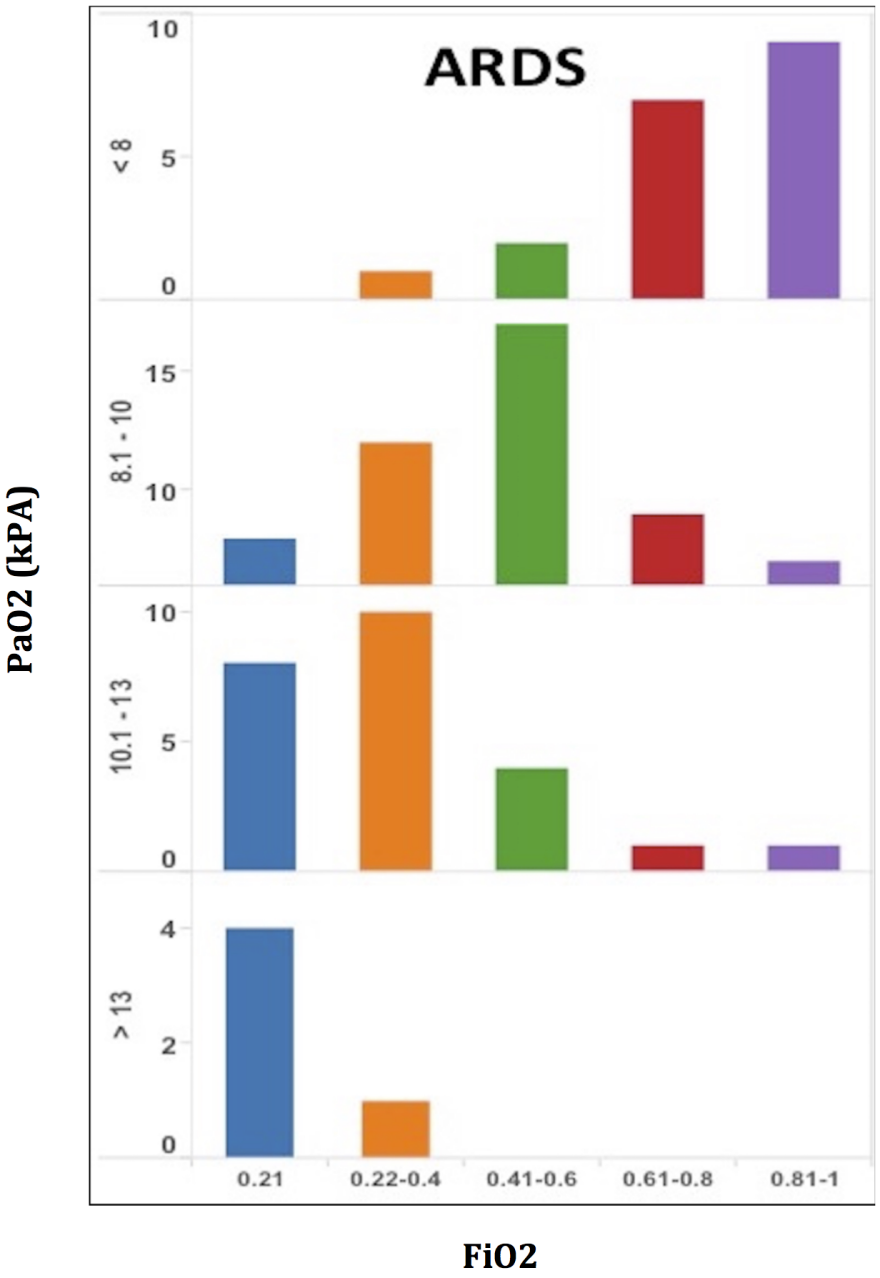


Figure 3.18: Target PaO_2 and FiO_2 Profile in Cardiac Arrest

The profile of PaO_2 (y-axis) and FiO_2 (x-axis) targeted in a child following cardiac arrest and moderate ventilatory requirements. The PaO_2 ranged from <8 kPa in the top panel to >13 kPa in the bottom panel. FiO_2 ranged from 0.21 (blue) through to 0.81-1 (purple). The pattern is of more restrictive PaO_2 targets with increasing FiO_2.

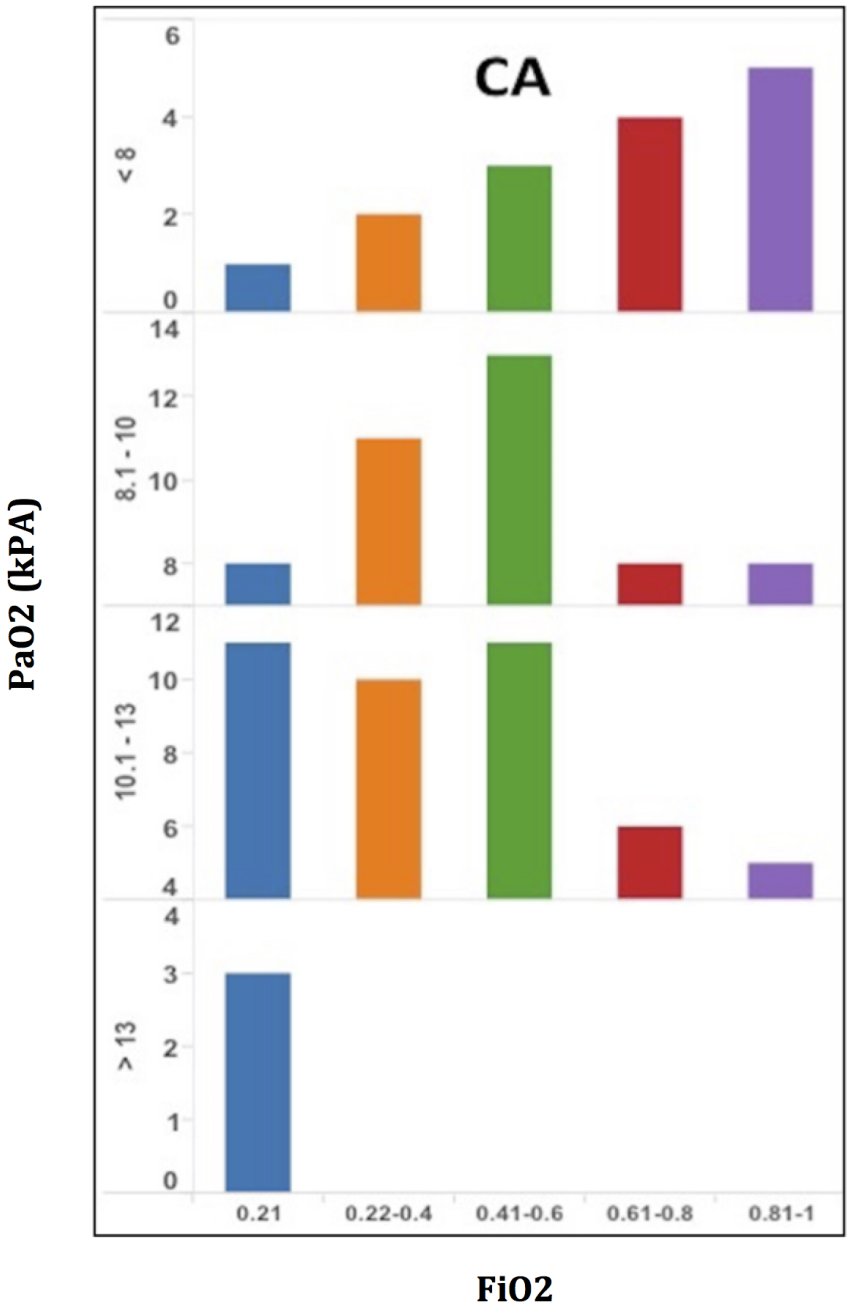


Figure 3.19: Target PaO_2 and FiO_2 Profile in Sepsis

The profile of PaO_2 (y-axis) and FiO_2 (x-axis) targeted in a child with sepsis and moderate ventilatory requirements. The PaO_2 ranged from <8 kPa in the top panel to >13 kPa in the bottom panel. FiO_2 ranged from 0.21 (blue) through to 0.81-1 (purple). The pattern is of more restrictive PaO_2 targets with increasing FiO_2.

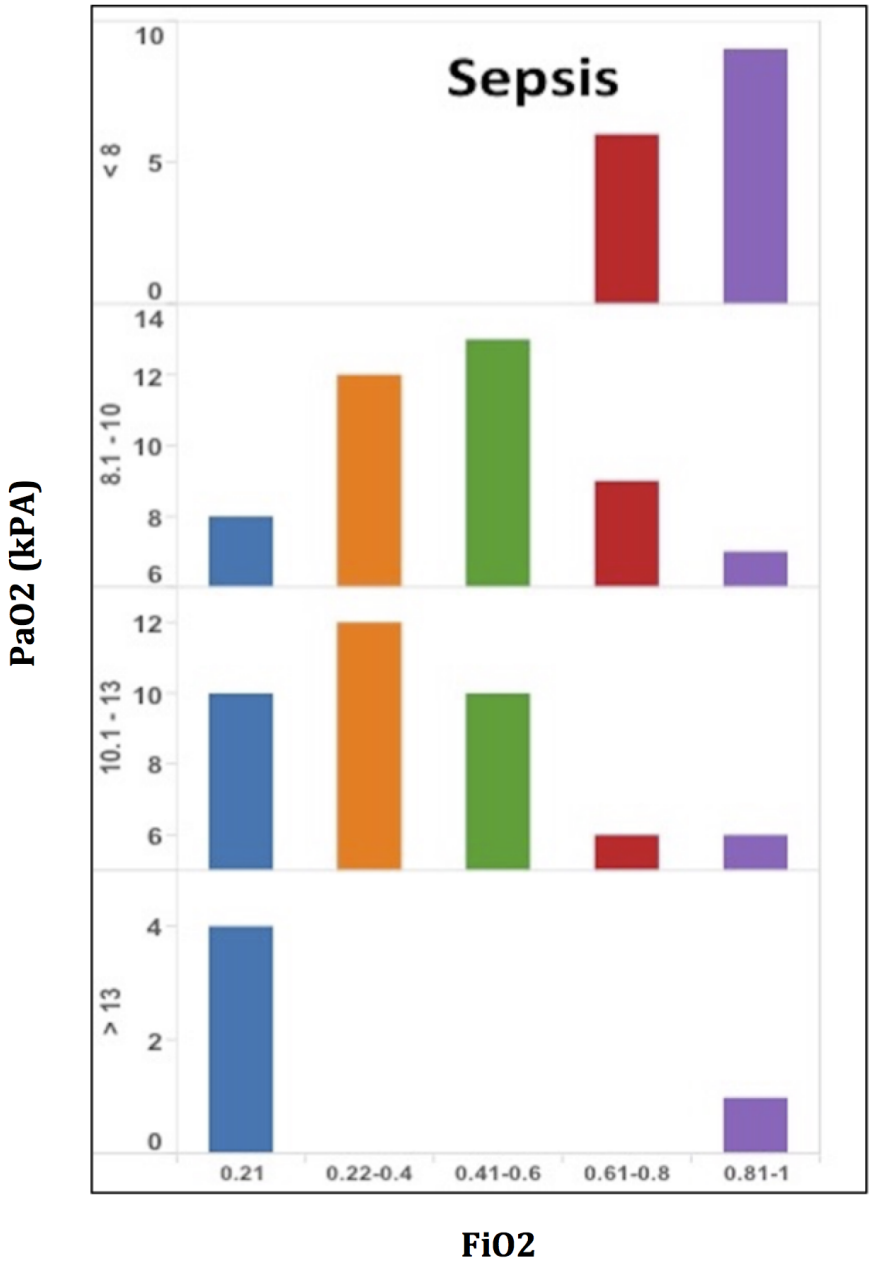


Figure 3.20: Target PaO_2 and FiO_2 Profile in Traumatic Brain Injury

The profile of PaO_2 (y-axis) and FiO_2 (x-axis) targeted in a child following traumatic brain injury and moderate ventilatory requirements. The PaO_2 ranged from <8 kPa in the top panel to >13 kPa in the bottom panel. FiO_2 ranged from 0.21 (blue) through to 0.81-1 (purple). The pattern is of liberal PaO_2 targets despite increasing FiO_2.

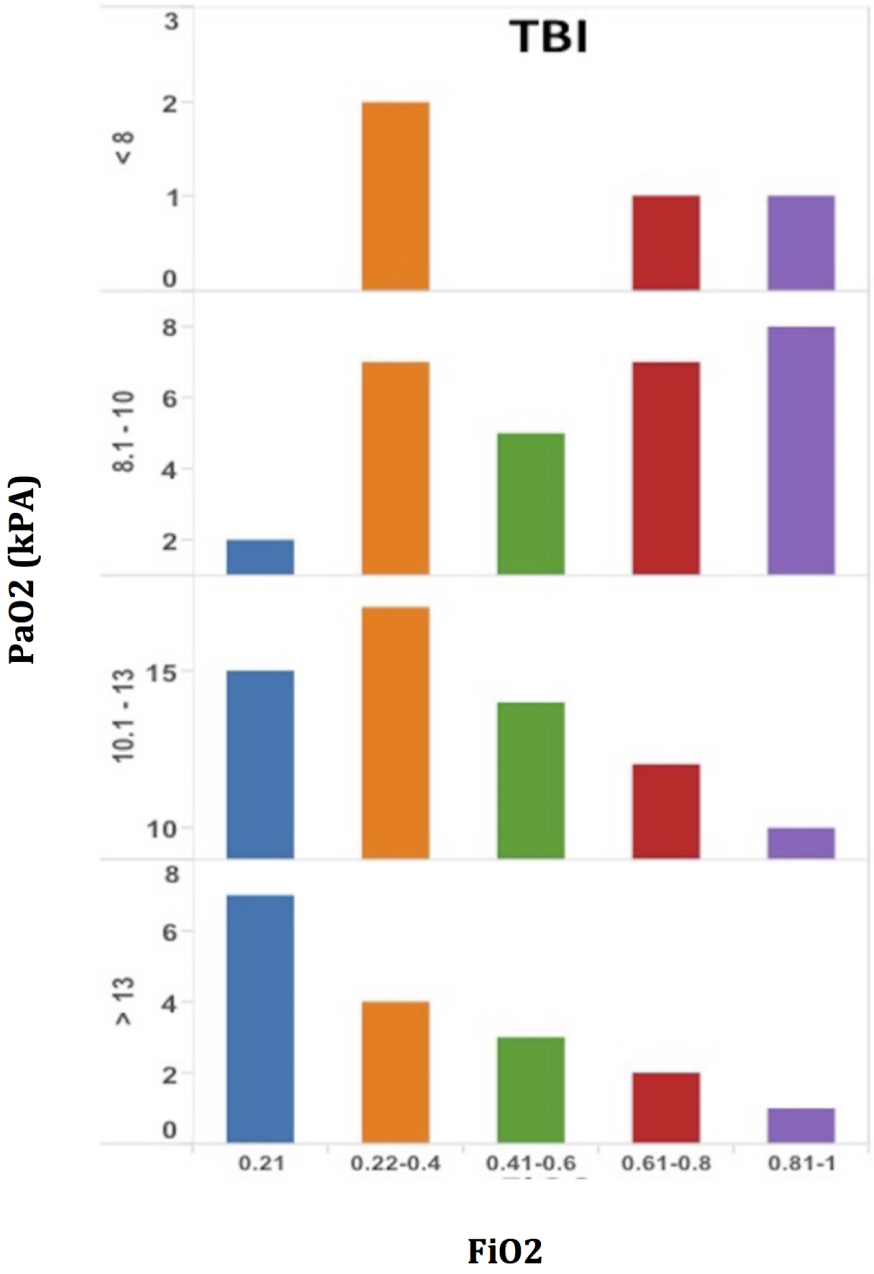
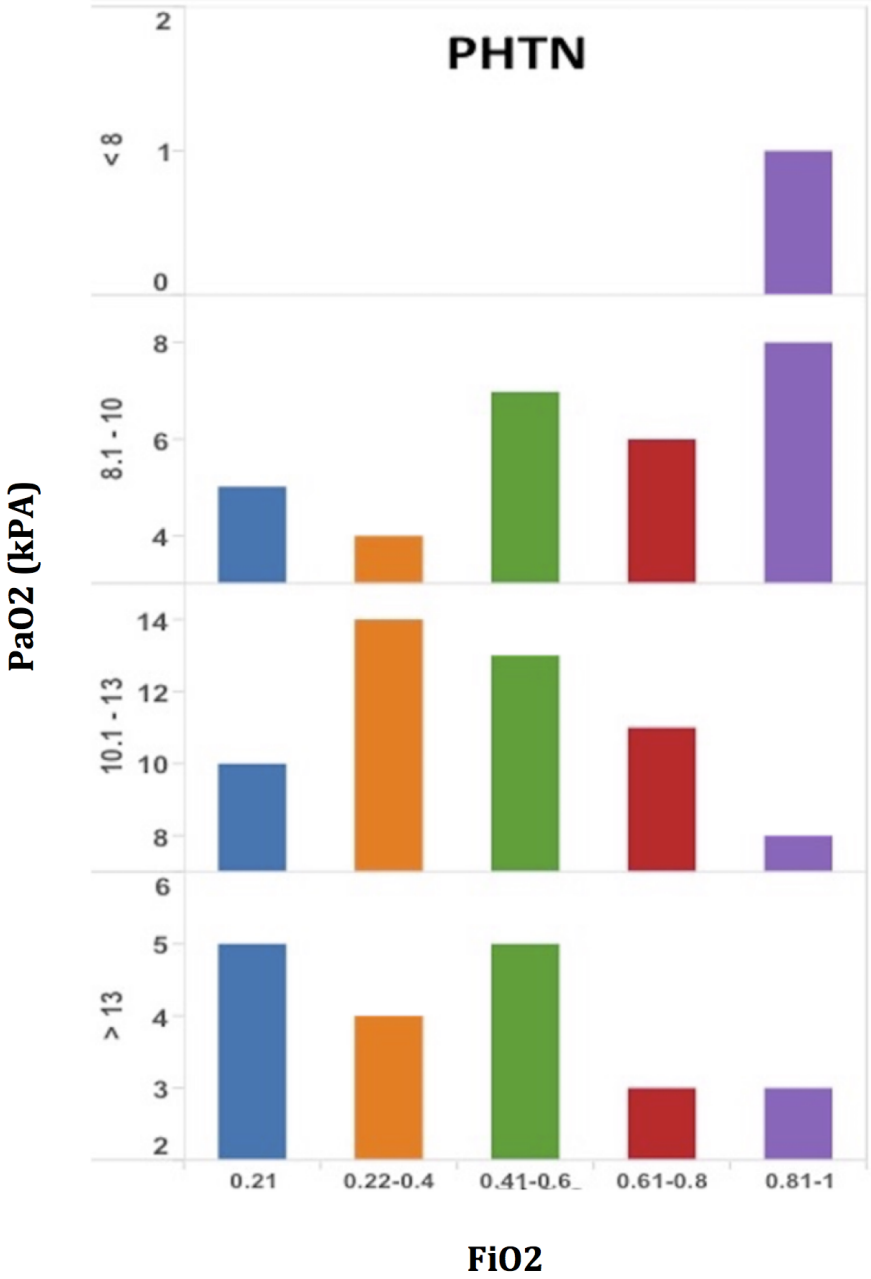


Figure 3.21: Target PaO_2 and FiO_2 Profile in Pulmonary Hypertension

The profile of PaO_2 (y-axis) and FiO_2 (x-axis) targeted in a child with pulmonary hypertension and moderate ventilatory requirements. The PaO_2 ranged from <8 kPa in the top panel to >13 kPa in the bottom panel. FiO_2 ranged from 0.21 (blue) through to 0.81-1 (purple). The pattern is of liberal PaO_2 targets despite increasing FiO_2.

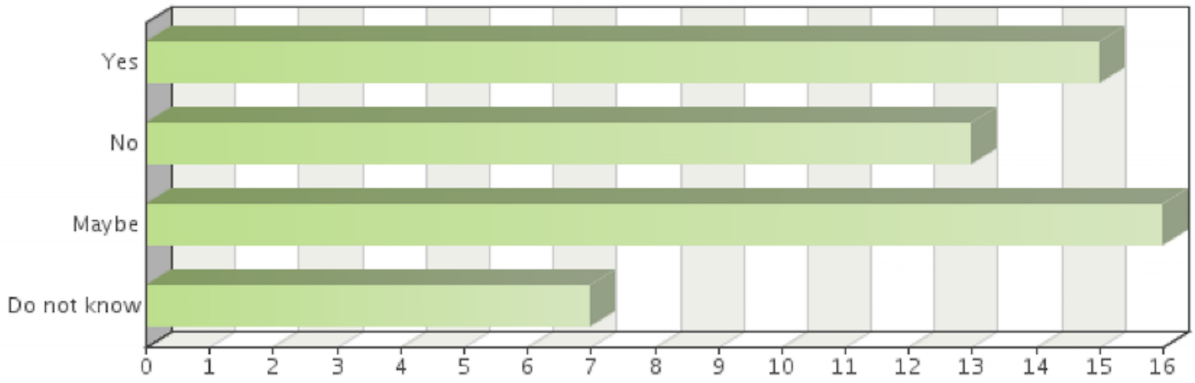


The response to no improvement after 24 hours of intensive care followed a similar trend as the initial management. A larger proportion of respondents targeted higher PaO_2 in children who suffered from pulmonary hypertension and TBI. The majority of respondents were permissive with PaO_2 targets for the children suffering from acute respiratory distress syndrome. Almost 58% of the respondents aimed for a FiO_2 of 0.4-0.6 to the amended PaO_2 targets.

An equal number of respondents felt there was a need for a randomised control trial with tight arterial oxygenation targets. A large number were not fully convinced of the need for a trial.(Figure 3.8) This suggests that clinicians are not yet convinced by the evidence of harm from hyperoxia in critical illness. A small proportion of the respondents felt it was not ethical to perform such a trial (11%).

Figure 3.22: Randomised Control Trial with Tight Arterial Oxygenation

The respondents answered the question: 'Is there a need for a randomised controlled trial exploring the restrictive oxygenation targets to normal targets?' The opinion is nearly equally split between the groups.



3.7 Conclusions

Despite the universal use of supplemental oxygen therapy in intensive care, its effect on the PaO_2 and the eventual outcome is unclear. Results of the systematic review suggest that hyperoxia may not have a significant effect on mortality in the overall group of critically ill children. The odds of death with hyperoxia seemed to increase (not statistically significant) in children admitted following cardiac arrest. This is consistent with the reports from adult studies. [11] The review suggests that avoiding hypoxia may be beneficial in all critically ill children, particularly in the TBI subgroup.

According to the review, hypoxia is harmful. This contradicts the hypoxic adaptation hypothesis. However, there was significant heterogeneity in the studies. Children admitted following a TBI seem to fair worse when hypoxic. Ong reports poor outcome in children <15 years of age following TBI (OR: 7.48, 95%CI: 1.27-21.76) in the presence of hypoxia. The composite outcome measure (poor outcome) was severe disability, persistent vegetative state or death at discharge and 6 months adjusted for Glasgow Coma Scale, subarachnoid haemorrhage, diffuse axonal injury and brain swelling. [221] Mayer performed a prospective observational study of 200 children with severe TBI. They observed a significantly higher mortality (55% vs. 7.7%) in those children with any of the following: hypotension, hypercarbia or hypoxia, compared to those without any of these signs. [222] The review findings seem to lend support to the international recommendations on the management of TBI in children. [223] The high heterogeneity in CA and the LRTI subgroups makes the interpretation of these results difficult.

Several limitations restrict the scope of this review. The number of studies included is small. Furthermore most were retrospective. The characteristics of the studies vary and the possibility of selection bias within each study is high. The funnel plot of the hyperoxia studies suggests some reporting bias.

However, the leave-one-study-out analysis suggests that the different outcome measures used in the studies do not affect the overall result of the meta-analysis. In

addition, the subgroup analysis accounts for some of the heterogeneity. The threshold of PaO_2 >200 mmHg (26.7 kPa) does not seem to be different to PaO_2 >300 mmHg (40 kPa).

Nearly all the studies explore the relationship of PaO_2 either <60 mmHg (8 kPa) or >300 mmHg (40 kPa) on mortality. This suggests an inherent bias in the setting of the PaO_2 thresholds for the cohorts. In addition, none of the studies have addressed the range in between 60 and 300 mmHg (8-40 kPa) as a continuous variable. Most of the studies included in this review have a small sample size. This further limits the generalizability of the results.

The extreme hypoxia observational study shows that extreme hypoxia is not a good predictor of mortality. This is in keeping with a case report from more than 40 years ago. [9] Even though I observed survival in the extreme hypoxia group, the functional outcome of these children was not investigated. With the small number of patients and incomplete assessment of outcome, it would be prudent not to draw firm conclusions from this finding. There was no difference in survival between children with and without a cardiac disease (combined cyanotic and acyanotic heart disease data). Unfortunately, the specific data on cyanotic cardiac disease patients were not available. It would be interesting to investigate if of

the cardiac children who survived, what proportion had a cyanotic cardiac disease.

The PaO_2 - mortality continuous variable study shows a 'U-shaped' relationship between PaO_2 and mortality in critically ill children. The risk of death predicted by the PIM2 and PIM3 models has an inverse law link to PaO_2. As the PaO_2 increased, the difference between SMR and mSMR ceased to exist. Thus, the influence of PaO_2 on PIM2 predicted risk of death is higher in the hypoxic range than the hyperoxic range.

The U-shaped relationship between PaO_2 and mortality observed in my study differs from the inverse relationship between PaO_2 and mortality in the PIM2 model. There may be two explanations to this apparent discrepancy. Although hyperoxic children seem to have a higher crude mortality, the relationship may not be causal. They are hyperoxic because clinicians recognise them as being sick and are reluctant to wean oxygen. Alternatively, PIM2 may need to have a modified coefficient for hypoxia and hyperoxia. There might be several reasons for the higher predicted mortality in the patients with hyperoxia such as brain death or other high risk diagnoses. These seem to be associated with death despite adequate oxygenation.

The survey results suggest variations in oxygen delivery practices. The higher response rate from the consultants is perhaps linked to their experiential knowledge. Of particular note, no oxygen weaning protocol exists in nearly three-quarters of

the PICUs.

The review suggests that a strategy that aims for normoxia and avoids hyperoxia in the CA subgroup may be better for survival. The variability in the TBI group is too great to allow firm conclusions to be drawn with respect to hyperoxia.

Limitations of the systematic review:

1. The literature search was conducted by a single author. Formal Cochrane reviews are reviewed by two independent authors. To account for this limitation, the search strategy was reviewed and ratified by an Institute of Child Health librarian.

2. There were no randomised control trials in the area of interest. Of the cohort studies that were included, risk of a selection bias was high. A thorough assessment of bias was performed with the Newcastle Ottawa Scale and funnel plots.

3. The co-variates included in the individual studies vary. This limits the significance of the odds ratio of the overall population. Where available, adjusted odds ratio was utilised for the meta-analysis.

4. The retrospective design and heterogeneous patient population with different mechanisms for hypoxia or hyperoxia make interpretation of the results difficult. The focus was on the relationship between PaO_2 and mortality. While PaO_2 is a marker of alveolar gas exchange, tissue oxygen delivery is influenced by various factors, including haematocrit and vasomotor tone. The effect of ventilatory strat-

egy on PaO_2 was not explored. The mode of death and withdrawal of care due to ongoing hypoxia may introduce bias to the association. However, despite these limitations, my study would inform future studies in this area.

Limitations of the observational studies:

The data for the two retrospective observational studies was sought from the PICANet team in Great Ormond Street Hospital. The team collect a large admission dataset, check for errors in data input data at the bedside and submit it to PICANet. Despite this process, there is a risk of incorrect data being sourced from the PICU clinical database.

Limitations of the survey:

1. As with any survey, the respondents are a self-selecting population. They include motivated individuals or people who already hold a strong opinion - both positive and negative - on the subject.

2. There were more doctors in the respondents. This may be partly due to the membership of the PICS. The ideal would be to sample a larger population with an equal proportion of professionals.

3. Unfortunately, I did not survey practitioners from neonatal intensive care units in the UK. As they have a different patient profile, it will be a valuable review that could be undertaken in the future. This is particularly pertinent in view of the reports from the Boost 2 and COT trials. These have shown that the restrictive

oxygenation targets cause worse outcom in premature infants. [224] [225]

Chapter 4

Oxygen Consumption in Children With a Suspected Mitochondrial Disorder

This chapter investigates the use of a novel biomarker - Near infra-red spectroscopy with a vascular occlusion test (NIRS VOT) to estimate oxygen consumption from forearm muscle. The NIRS VOT method has been described in detail in chapter 2 (section 2.1). Children with suspected mitochondrial disease and other neurogenetic disease were chosen to test the usefulness of this technique.

4.0.1 Rationale for Choosing Mitochondrial Disease Patients

With advances in science, numerous health conditions have been attributed to mitochondrial dysfunction. The majority of these are due to defects in the respiratory electron transport chain. [226] These manifest as an inability to utilise oxygen. Phenotypically they demonstrate exercise intolerance, collapse in the neonatal period with lactic acidosis, seizure disorders, neurodevelopmental regression or as a neuromyopathy. [227]

Taivassalo et al demonstrated an increase in the partial pressure of oxygen in venous blood in patients with mitochondrial myopathy following a forearm exercise. Furthermore, they found a linear correlation between the rise in partial pressure of venous oxygen and severity of oxidative impairment (peak systemic $a - vO_2$ difference) as assessed during exercise. [228] By extrapolating from this inability to extract oxygen, researchers have devised a test to assess the severity of mitochondrial myopathy. The modified exercise test (MET) aids in the screening of these patients by measuring the rise in lactate following a sub-anaerobic exercise test. The MET showed a sensitivity of 78% and a specificity of 100% when compared to healthy controls. [229] In another set of experiments, Taivassalo et al showed that the oxygen delivery during exercise is increased in quadriceps muscle in mitochondrial myopathy patients. In addition, the capillary density is increased,

suggesting a localised response to inefficient oxygen utilisation at the respiratory electron transport chain. [230] The concept of mitochondrial disease physiology is that the impact of the deficiencies of respiratory chain enzymes is a reduced oxygen metabolism and energy production. These studies support this concept and the reduced oxygen metabolism seems to match the clinical picture.

In this thesis, I wished to investigate the utility of NIRS in critical illness and, as defended above, congenital mitochondrial disease group provided a valuable potential positive control of reduced oxygen utilisation. However, I have not made a direct comparison between mitochondrial disease and critically ill children. I looked at the mitochondrial disease children when well so that critical illness was not a confounding factor. The aim was to look at a pure mitochondrial disease state.

4.1 Introduction

Mitochondrial disease is a term used to describe a heterogeneous group of inherited conditions with a defective oxidative phosphorylation as the central process. They are rare. The birth prevalence of mitochondrial disease in the western world is estimated at 1 in 5000. [231] [232] The most common mitochondrial diseases (both clinical and biochemical phenotypes) are leigh syndrome, Kearns-Sayre disease and complex IV disorders (Respiratory electron transport chain protein - discussed in

section 1.8.1). [112]

Typical presentations of severe mitochondrial disease include a delay in reaching developmental milestones or an acute admission to the emergency department with metabolic acidosis (typically associated with hyperlactatemia) and some combination of encephalopathy, myopathy or a multi-system disorder. The family history may include consanguinity, early miscarriages, sudden unexpected deaths or an affected relative. Making a diagnosis usually requires several invasive blood, urine and cerebrospinal fluid tests, muscle and skin biopsies. A final diagnosis may take months or, in a significant proportion of cases, may not be confirmed.

The varying symptomatology makes applying strict criteria for diagnosis difficult. Some clinicians employ a set of major and minor criteria to assist in the diagnosis of mitochondrial disease. These incorporate the clinical phenotype with the histology, functional characteristics, molecular and enzymology studies from muscle and skin biopsies. [233]

Mitochondrial diseases are rarely treatable. The few reported exceptions include the riboflavin transporter defect, in which riboflavin supplementation can slow disease progression and occasionally improve the clinical condition. [234] Coenzyme Q_{10} (CoQ) acts as an electron carrier between Complex I/II and substrates from fatty acid beta oxidation and Complex III. When given as a supplement, it is beneficial in patients with primary CoQ deficiency but probably not helpful in

Complex IV disorders (Cytochrome C Oxidase - COX). [235]

The follow-up of a mitochondrial disease patient ideally should include objective serial measurements of their functional status. However, there are no universally agreed validated scoring systems and those that have been proposed are complicated and cumbersome and difficult to complete in a busy outpatient clinic setting. [236] [237] There is no bedside 'gold standard' test to diagnose and monitor mitochondrial disease either. In summary, the diagnosis, prognostication and objective measure of treatment effect are all challenging in mitochondrial disease management.

The predominant site of oxygen metabolism in a cell is the mitochondrion. [238] Therefore, a measure of the oxygen consumption could provide insight into mitochondrial function. NIRS VOT is a candidate for estimating oxygen consumption and may aid in quantifying this function. The principle of NIRS has been detailed previously in section 1.11. Wilson utilised NIRS with arterial occlusion to measure oxygen consumption in patients with heart failure. [239] Other investigators were able to differentiate between normal subjects and mitochondrial myopathy (MM) patients using NIRS with a treadmill exercise protocol. [240] Van Beekvelt studied 5 adult patients with MM with a vascular occlusion test (VOT) at rest. They recorded a VO_2 of 0.063 ± 0.027 ml/min/100 grams ($mean \pm SE$) in patients with MM as compared to 0.106 ± 0.006 ml/min/100 grams in normal adult volunteers

(p=0.02). [241] Grassi performed NIRS VOT in a similar group of patients. They demonstrated a statistically significant difference in the $mean \pm SE$ peak VO_2 between the MM patients and controls. [242] These results support the utility of NIRS VOT as a possible tool in the diagnosis of mitochondrial disease. More recently, in a randomised controlled trial, Glover used NIRS as a tool to measure the change in oxygen consumption with supplementation of Coenzyme Q_{10}. They found no difference in the oxygen saturation at rest, average saturation and maximal desaturation between the treatment groups after a 90-s exercise protocol. [243]

Magnetic resonance spectroscopy (MRS) is another technique that is employed in the assessment of mitochondrial disease. It is a non-invasive method that aids in screening of patients. Furthermore, it is able to assess mitochondrial function in-vivo. The principle is to use a radiofrequency pulse to excite nuclei placed under high power magnetic conditions. The nuclei absorb energy and are displaced from their thermal equilibrium. The nuclei release this energy when they relax back to equilibrium. Each chemical in the tissue (brain or muscle) has an identifying signature response in the MR spectrum. For example, abnormal oxidative metabolism in the mitochondrial present as a change in the high-energy phosphate levels in the MR spectrum. This can be extrapolated to tissue oxidative function [244] Matthews et al performed resting muscle MRS in mitochondrial myopathy pa-

tients (n=17, age range 1-73 years) with mild clinical signs. They chose other myopathy patients (no-mitochondrial) as controls. They were able to complete the test in 30 minutes. They noted altered phosphorus metabolite concentrations suggestive of impaired cellular energy metabolism. In addition, results correlated well with clinical signs. [245] I was unable to employ this technique as it is not a bedside measure, more expensive and needs an established experimental MRI research service. In addition, previous studies suggest that my patient group would have had similar results as the NIRS VOT with a MRS technique. Therefore, for this thesis I worked with NIRS VOT.

Tissue oxygen index (TOI) was measured with Near infrared spectroscopy vascular occlusion test (NIRS VOT) as described in chapter 2.1. The difference in tissue oxygen index between the lowest value of tissue oxygen index (at the end of blood pressure cuff inflation) to the baseline is termed Drop TOI. This may be an indirect measure of tissue oxygen consumption.

The similarity between the mitochondrial disease patient and the patient on intensive care is the reduced oxygen consumption. [246] The discussion above supports the theory that mitochondrial disease patients have restricted oxygen utilisation. In comparison, despite adequate oxygen delivery, the intensive care patient might

be limited in his capacity to consume oxygen. This limitation could result in physiological responses as described in chapter 1 (section 1.5). By investigating the mitochondrial disease patients, not only am I able to validate the NIRS VOT technique, but also start understanding the adaptive mechanisms that might act in the chronic hypoxic setting. Further, this project augments my understanding of the mitochondrial respiratory chain and its function. I was able to utilise this knowledge in my gene expression study detailed in chapter 6. It focuses on the electron transport chain and explores mitochondrial dysfunction in the intensive care patient.

The work described in this chapter is set out to assess if NIRS VOT values differ between mitochondrial disease children and healthy controls. Furthermore, the utility of this technique as a diagnostic tool in children with suspected mitochondrial disease was explored by comparing them to children with other neurogenetic diseases (non-mitochondrial disease).

4.2 Methods

General objectives:

1. To assess the utility of NIRS VOT for detecting differences in forearm muscle tissue oxygen consumption in children with a known mitochondrial disease and

2. To assess the potential of NIRS VOT as a screening tool to identify patients

with a higher probability of having a mitochondrial disease.

Hypothesis 1: Mean Drop TOI in cases of confirmed mitochondrial disease will be equivalent to controls (neurogenetic disease and healthy children).[Null hypothesis]

Hypothesis 2: Cases with a suspected mitochondrial disease with mean Drop TOI below a threshold value have an increased risk of having confirmed mitochondrial disease (by either muscle enzymology or genotype). [Alternative hypothesis 1]

Hypothesis 3: NIRS VOT results will not change the probability of a diagnosis in children with suspected mitochondrial disease. [Alternative hypothesis 2]

Design: Prospective observational study.

Ethics approval:

The project was registered with the R&D office in Institute of Child Health (R&D ref: 13AR09). The Camden and Islington Research Ethics Committee (REC ref:13/LO/1259) approved the study on 4th October 2013. The final R&D approval was gained on 28th November 2013.

The tests were performed after informed written consent was obtained from the parents. Children, where able, signed an 'Assent form'. A signed copy of the consent form, parent information sheet along with the age-specific child information sheet was given to parents. The original copy of the signed consent form was stored in the research office in the paediatric intensive care unit.

Inclusion criteria:

All patients with a suspected mitochondrial disease who were referred to Prof Shamima Rahman were eligible for inclusion. Both inpatients and outpatients were considered for recruitment. The inpatient cohort was limited to elective admissions because of the potential effect of acute illness on mitochondrial function. Disease controls with non-mitochondrial neurogenetic diseases were recruited from Dr DeVile's paediatric neurology outpatient clinic. These patients do not have a primary mitochondrial disease. The prevalence of secondary mitochondrial dysfunction in this group is unknown.

The procedure was discussed with all eligible patients including those who suffer from dystonic movements and muscle pain. The decision to undergo the test (after formal consent) was made if the parents anticipated that the test would not cause an increase in dystonia or cause undue pain.

Exclusion criteria:

1. Children admitted to the ward following unexpected deterioration

3. Children with a cyanotic congenital heart disease

4. Ex-premature infants (<30 weeks gestation at birth)

Inpatients:

In-patients with a suspected mitochondrial disease are usually admitted to the neurology and metabolic wards in Great Ormond Street Hospital (GOSH). When a child's admission was planned, either the nurse in-charge of the ward or the clinicians informed me. Permission to approach the family was obtained from the lead clinician.

Following this, an invitation letter, information sheets and a consent form were sent to the family. The documents were sent at least 1 week before the planned date of admission. The family was approached on the day of admission and any questions about the study answered. The families were free to withdraw consent at any time.

Outpatients:

Eligible out-patients:

Children (<16 years) with a follow-up in the outpatient department at Great Ormond Street Hospital.

A list was created of patients meeting inclusion criteria 2 weeks prior to the outpatient appointment. Families received an invitation letter, information sheets and a consent form at least 1 week in advance. They were informed that they would be approached on the day of clinic. If they were unwilling to participate, the family had no further involvement in the study.

Participants were classified as outlined in Table 4.1.

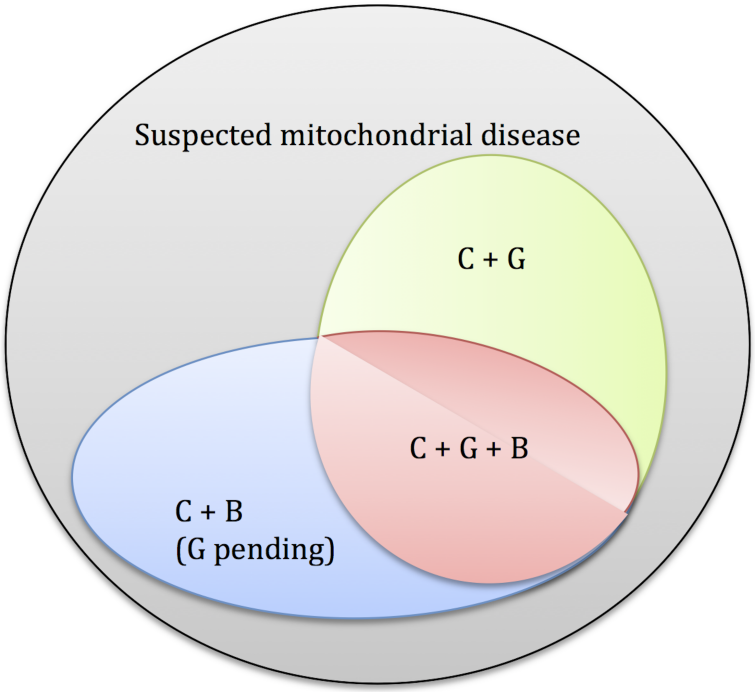
Table 4.1: Classification of children recruited in this study

Group	**Diganosis**
Controls	**Neurogenetic disease:** Non-mitochondiral Non-biochemical abnormality Neurological abnormality
	Healthy controls: Healthy children
Mitochondrial diease group	Genetically confirmed (and/or)
	Biochemically confirmed with good clinical phenotype (and/or) Non-mitochondiral genetic abnormality and possible secondary mitochondrial dysfunction

The 'textbook' mitochondrial disease patient would have a strong clinical phenotype, a biochemical defect (as measured from muscle biopsy) and a known genetic mutation. But methods and technology for genetic analyses are still evolving and nearly 50% of the patients with the phenotype and biochemical defect do not currently have a genetic diagnosis. Less frequently, patients have normal biochemistry in the setting of a strong clinical phenotype and/or a known mutation. The Venn diagram (Figure 4.1) represents the subsets: Patients with a strong clinical phenotype, patients with a genetically confirmed and/or a biochemically confirmed diagnosis. The aim of this project was to investigate if the NIRS VOT differentiates between patients within the Venn diagram and those outside it.

Figure 4.1: Venn Diagram Showing Inter-relationship of the Subsets Within the Suspected Mitochondrial Disease Group

The children recruited into the mitochondrial disease group all were suspected to have mitochondrial disease. They could be further subdivided into four subsets: 1. Children with a strong clinical phenotype (C) in addition to a genetic mutation (G) (pastel green shaded area), 2. Children with a strong clinical phenotype (C) and biochemical abnormality (B) (blue shaded area), 3. Children with a strong clinical phenotype (C), a genetic mutation (G) and a biochemical abnormality (B) (pink shaded area) and 4. Children who do not fall into any of the preceding categories (grey shaded area).



The recruits underwent two tests - the NIRS VOT and a cardiac output measurement (with Ultrasound cardiac output monitor). The full details of the techniques are described in chapter 2 (section 2.1 and 2.2).

4.3 Statistical Analysis

The sample size was calculated using two methods:

1. Previous work with NIRS in a study of adults with one clinical syndrome of a mitochondrial disease (Chronic Progressive External Ophthalmoplegia) observed a mean fall in the tissue oxygen index of 0.106 (se=0.006) in controls and 0.063 (se=0.027) in chronic progressive external ophthalmoplegia patients.[12] If this same percentage difference (37%) were to be reproduced in this study, then with an alpha set at 0.05 and power at 80%, 16 patients would be required in each group.

2. To distinguish a case of mitochondrial disease from non-mitochondrial disease cases, one requires estimates of the mean and variance of TOI from non-mitochondrial disease cases. These have not been reported in the literature previously. We, therefore, made an assumption that the neurogenetic disease (non-mitochondrial disease) group may behave in a similar fashion to normal children. Therefore for the sample size calculation, pilot data from children with mitochondrial disease and data from normal children in the Young Everest Study 2 (chapter 5) were used. The pilot data from mitochondrial disease children has been included in the final data analysis.

The sample size calculation using two means (mitochondrial disease group and healthy children) from the pilot study yielded a sample size of 28 per group. The

mean for group 1 (mitochondrial disease) was 13.2 and that for group 2 (healthy children) was 21.1. A second method was used to calculated sample size using a two-sample two-sided equality analysis. The power was set at 80, alpha set at 5, standard deviation of the mitochondrial disease group was 7.61 and standard deviation was healthy children of 7.09. The formula includes the ratio between sample sizes of two groups. In my case, this is equal to 1. The result is 13 per group. I chose to recruit a larger sample size as per the first calculation.

Numerous additional factors introduce imprecisions into NIRS estimations of muscle oxygen index. These include variability in patient size, muscle mass and severity of underlying mitochondrial disease. The aim was to recruit 30 patients in each group to account for these factors.

The Drop TOI (difference between baseline and lowest Tissue Oxygen Index) of the mitochondrial disease group was compared to healthy controls and then to the neurogenetic disease group. The recordings in children with suspected mitochondrial disease had significant movement artefact. Therefore, summary measures were utilised for analysis. The reference measurement to confirm mitochondrial disease was either genetic or biochemical results.

The following were addressed:

1. To test the null hypothesis i.e the mean DropTOI is not different between children with mitochondrial disease and controls (neurogenetic disease and healthy children), a 't-test' was used to compare the groups.
2. To test alternative hypothesis 1: A 2x2 table was created. Proven mitochondrial disease (yes or no) was tabulated against DropTOI threshold value (e.g., $\geq$14 or <14). Thresholds were selected by interrogating the TOI values of controls and mitochondrial disease patients and choosing a level that had no or minimal crossover. Fisher's exact test was used to compare those with mitochondrial disease to those without.
3. To test alternative hypothesis 2 i.e. NIRS VOT results will not increase the probability of a diagnosis of mitochondrial disease: Bayesian inference with a Fagan's nomogram was employed.

Bayesian statistics involve applying previous theoretical and empirical knowledge to formulate hypotheses, rank them on the basis of observed data and update prior probability estimates and hypotheses using observed data. [247] The prior probability of mitochondrial disease or 'prior' was determined from previous knowledge of the epidemiology of mitochondrial disease and referral patterns to the outpa-

tient clinic.

In the general population, the incidence of mitochondrial disease is 1 in 5000 (0.02%). However, children presenting to the outpatient clinic with clinical signs suggestive of a neuromuscular, neurogenetic or mitochondrial disorder are expected to have a higher probability than the general population. In addition, Prof Rahman's outpatient clinic is a specialist clinic for children with suspected mitochondrial disease. Other clinicians from within GOSH and the district general hospitals in the region refer patients to her when they suspect mitochondrial disease or have ruled out other more common diagnoses. This increases the 'prior' further.

After consideration of these parameters, a prior probability of mitochondrial disease was chosen from the proportion of children with mitochondrial disease and all children tested with NIRS VOT within this thesis. This yielded a 'prior' of 0.4. Expert opinion from other clinicians in the metabolic medicine and neurology department in GOSH were sought. The general consensus was that this 'prior' was a reasonable estimate. A Fagan Nomogram was employed with the agreed 'prior' to calculate the posterior probability or 'posterior'.

4.4 Results

Demographics:

The total number of children recruited was: 43 children in the mitochondrial dis-

ease group and 32 controls (12 healthy children from the sea level tests within the Young Everest Study 2, one healthy sibling of a patient with suspected mitochondrial disease and 19 children in the neurogenetic disease group).

The results are presented in two sections - Comparison of children with mitochondrial disease to controls and a further exploratory comparative analysis of the mitochondrial disease to neurogenetic disease group.

4.4.1 Section 1: Comparison of Mitochondrial Disease to Controls

The age, weight and height were not normally distributed (Table 4.2). Mitochondrial disease children were shorter than healthy controls. The median age, weight and mean/median cardiac index were not statistically different between mitochondrial disease group and controls.

Table 4.2: Characteristics of children with mitochondrial disease and controls

The values presented are median with interquartile range within brackets. CI - Cardiac index.

Characteristics	**Mitochondrial disease (n=43)**	**Controls (n=32)**	**p-value**
Age (yrs)	$10.4\ (6.5 - 13.6, 43)$	$10.0\ (7.8 - 13.8, 32)$	0.39
Weight (kgs)	$31\ (21.7 - 47, 29)$	$40\ (26.9 - 47, 29)$	0.31
Height (cm)	$135.1\ (117.4 - 150.4, 28)$	$146\ (126.2 - 160, 29)$	0.23
CI ($L/min/m^2$)	$3.1\ (2.5 - 4.3, 25)$	$3.0\ (2.8 - 3.8, 28)$	0.63

A linear regression with Drop TOI as the outcome measure with age and presence of mitochondrial disease as independent variables was performed. Although there was a decrease in Drop TOI by 0.05 for every year increase in age, this was not statistically significant.

Testing of the null hypothesis

The baseline TOI was not different between groups. The Drop TOI was not different between the mitochondrial disease group to controls (t-test, $p = 0.14$). The different sections of the NIRS VOT curve were also compared. Box plots were created to assess normal distribution. Figures 4.2 show that the data is not nor-

mally distributed. Therefore, a Mann-Whitney test was employed to compare the groups. (Table 4.3) The highest TOI was statistically different between the groups (p=0.02).

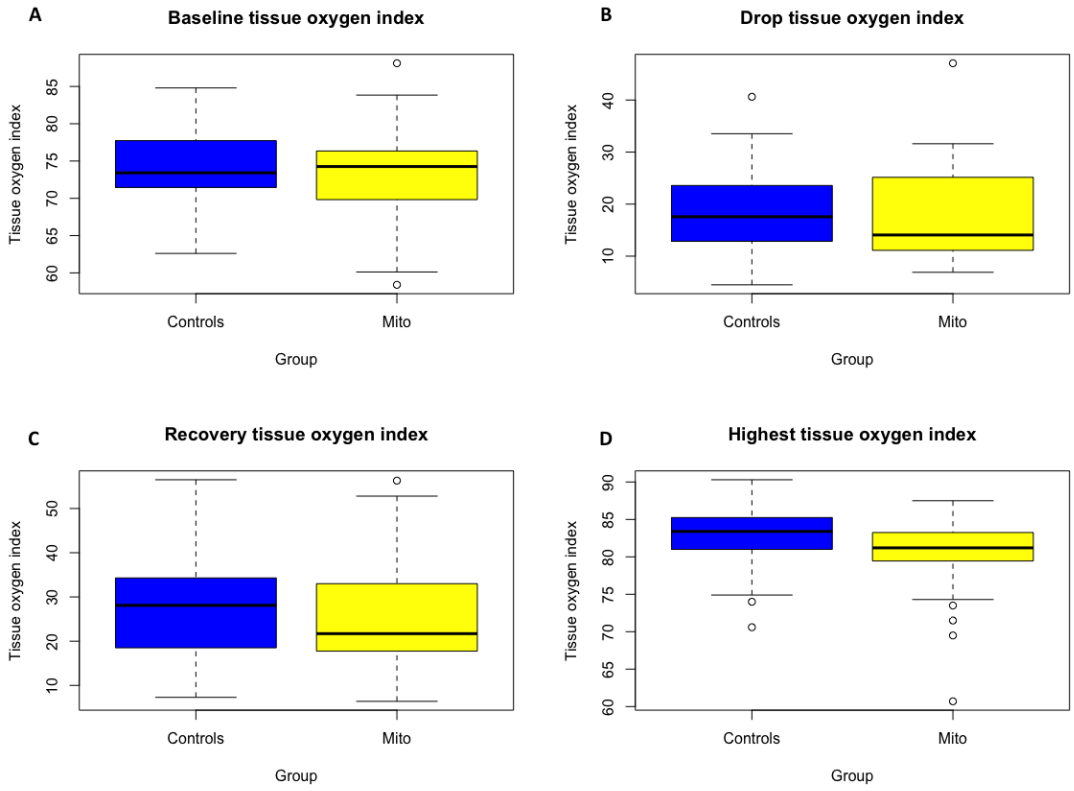
Table 4.3: Tissue oxygen index (TOI) values of children with mitochondrial disease and controls

The values presented are median with interquartile range within brackets

Characteristics	**Mitochondrial disease (n=43)**	**Controls (n=32)**	**p-value**
Baseline TOI	74.2 (69.8 – 76.3)	73.4 (71.5 – 77.6)	0.42
Drop TOI	14.0 (11.1 – 25.1)	17.6 (13.1 – 23.5)	0.44
Highest TOI	81.2 (79.4 – 83.2)	83.4 (81.4 – 85.1)	0.02
Recovery TOI	21.7 (17.7 – 33.0)	28.1 (18.6 – 34.1)	0.42

Figure 4.2: Box Plots of Various Sections of Tissue Oxygen Index Comparing Mitochondrial Disease Group to Controls

Panel A - Baseline Tissue Oxygen Index, Panel B - Drop Tissue Oxygen Index, Panel C - Recovery Tissue Oxygen Index and Panel D - Highest Tissue Oxygen Index. Blue box represents data from controls while yellow box is from the mitochondrial disease group. The dark black line in the middle of the box depicts the median value. The error bars represent 1st and 3rd quartile values. The small circles denote outlier values.



Testing of the alternative hypothesis 1

A Drop TOI value of 14 was noted to have the least crossover between the mitochondrial disease group and controls. This was set as the cutoff for the 2x2 table (Table 4.4).

Table 4.4: Comparison of children with mitochondrial disease to controls

Test	**Mitochondrial disease present**	**Mitochondrial disease absent**	**Total**
NIRS VOT Positive	19	9	28
NIRS VOT Negative	24	23	47
Total	43	32	75

The Fisher exact test statistic was 0.22 and showed that NIRS VOT is unable to differentiate between mitochondrial disease and controls.

Testing of the alternative hypothesis 2

The Fagan nomogram was employed to include the prior probability of mitochondrial disease. As described earlier, 0.4 was chosen as the 'prior'.

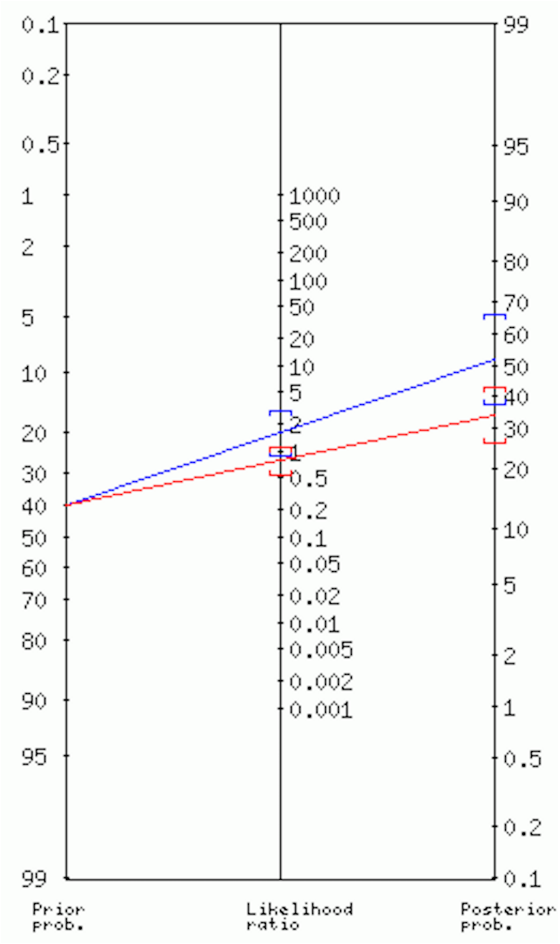
Table 4.5: Fagan nomogram posterior probabilities

Test	**Positive likelihood ratio**		**Posterior probability**		**Description**
Positive test	1.57	(95%CI:0.84-2.92)	51%	(95%CI:36-66%)	$\sim$ 1 in 2 children with positive test have the disease
Test	**Negative likelihood ratio**		**Posterior probability**		**Description**
Negative test	0.78	(95%CI:0.54-1.12)	34%	(95%CI:26-43%)	$\sim$ 1 in 1.5 children with negative test are well

Both the positive and negative posterior probability had wide confidence intervals. Therefore, NIRS VOT is unable to discriminate between mitochondrial disease and the control population.

Figure 4.3: Change in Probability of Diagnosis of a Mitochondrial Disease After Near Infrared Spectroscopy Vascular Occlusion Test (NIRS VOT) Compared to Healthy Children.

The blue line represents the change in posterior probability following a positive NIRS VOT. The red line shows the change in the posterior probability after the NIRS VOT is negative. Prior probability is set at 0.4.



4.4.2 Section 2: Comparison of Mitochondrial Disease to Neurogenetic Disease

This section explores the ability of NIRS VOT to differentiate between children with mitochondrial disease and other neurogenetic disease. Patient demographics - age, weight, height and cardiac index - were not different between the groups. Although the mitochondrial disease group were shorter and weighed less, these differences were not statistically significant.

Table 4.6: Characteristics of children with mitochondrial disease and neurogenetic disease group

The values presented are median with interquartile range within brackets. CI - cardiac index.

Characteristics	**Mitochondrial disease (n=43)**	**Neurological disease (n=19)**	**p-value**
Age (yrs)	$10.4\ (6.5 - 13.6, 43)$	$12.1\ (7 - 14, 19)$	0.48
Weight (kgs)	$31\ (21.7 - 47, 29)$	$41.1\ (25.2 - 51.6, 16)$	0.35
Height (cm)	$135.1\ (117.5 - 150, 28)$	$147.7\ (122 - 161.2, 16)$	0.58
CI (L/min/m^2)	$3.1\ (2.5 - 4.3, 25)$	$3.2\ (2.9 - 4.1, 15)$	0.64

Testing of Null hypothesis

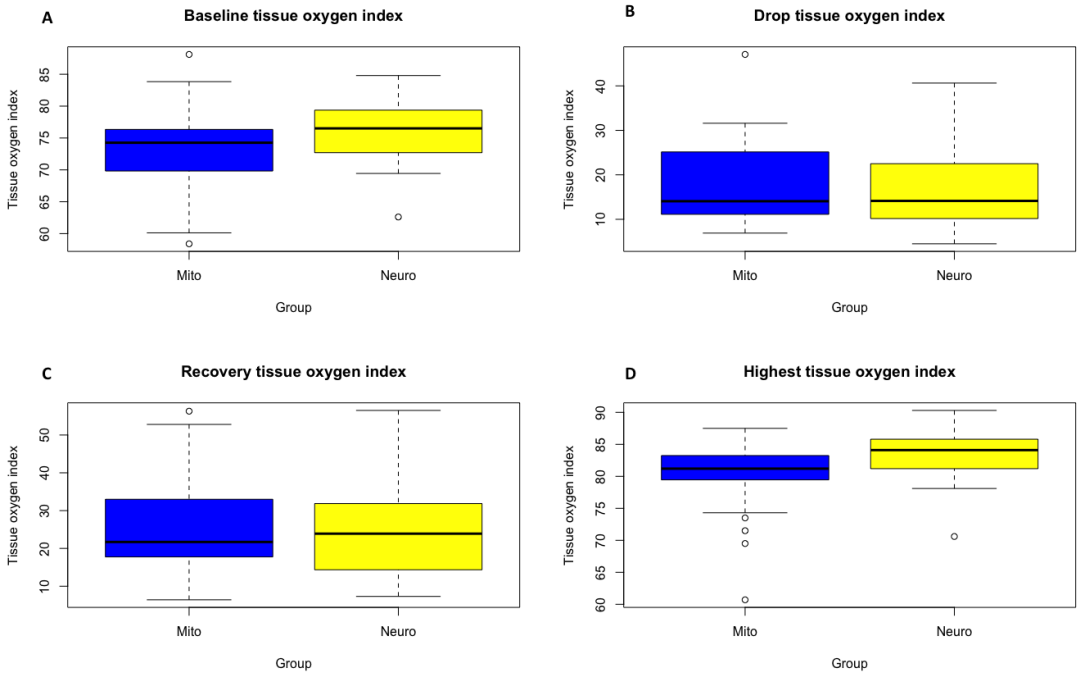
Figures 4.4 show that the data is not normally distributed. Therefore a Mann-Whitney test was employed to compare the groups. The baseline TOI between mitochondrial disease and neurogenetic disease group trends towards significance. The highest TOI was different between the groups ($p = 0.03$). This result is similar to the comparison of mitochondrial disease to controls. (Table 4.7).

Table 4.7: TOI values of children in mitochondrial and neurogenetic disease group The values presented are median with interquartile range within brackets.

Characteristics	**Mitochondrial disease (n=43)**	**Neurological disease (n=19)**	**p-value**
Baseline TOI	74.2 (69.8 – 76.3)	76.5 (72.7 – 79)	0.07
Drop TOI	14.0 (11.1 – 25.1)	14.1 (10.1 – 21.5)	0.77
Highest TOI	81.2 (79.4 – 83.2)	84.1 (81.2 – 85.6)	0.03
Recovery TOI	21.7 (17.7 – 33.0)	23.9 (14.3 – 31.0)	0.86

Figure 4.4: Box Plots of Various Sections of Tissue Oxygen Index Comparing Mitochondrial Disease Group to Neurogenetic Disease Group

Panel A - Baseline Tissue Oxygen Index, Panel B - Drop Tissue Oxygen Index, Panel C - Recovery Tissue Oxygen Index and Panel D - Highest Tissue Oxygen Index. Blue box represents data from mitochondrial disease group while yellow box is from the neurogenetic disease group. The dark black line in the middle of the box depicts the median value. The error bars represent 1st and 3rd quartile values. The small circles denote outlier values.



Power recalculation:

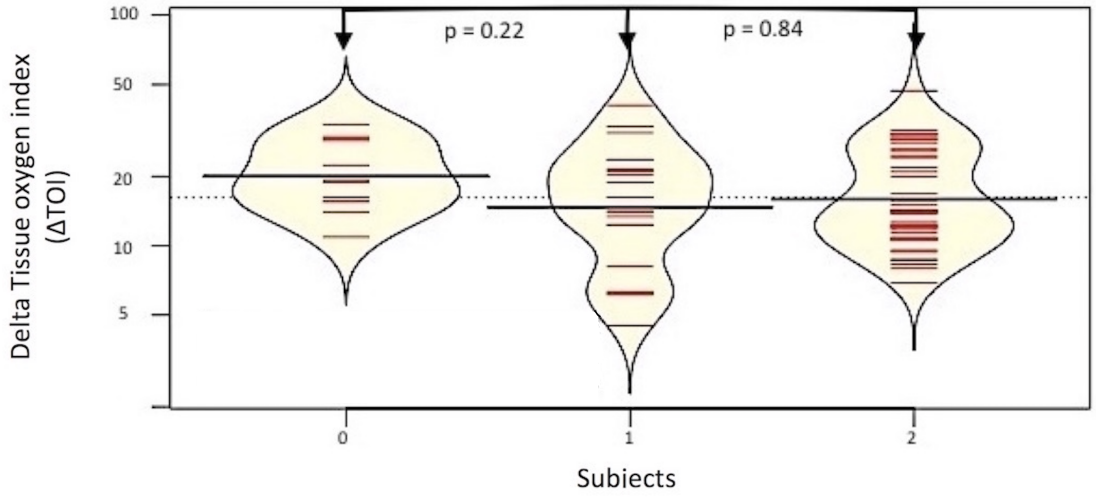
The power was recalculated after collecting 48 patients (40 disease group - Mitochondrial disease group and 8 disease controls - neurogenetic disease group). With a median for neurogenetic disease group as 22.19, median for mitochondrial disease group as 14.17 and a standard deviation of 8.76, a sample size of 40 and alpha error set at 0.05, the power would be 0.98. With these values, a power of

80% would be achieved with a sample size of 18. I recruited 11 more patients in the neurogenetic disease group and 3 further patients in the mitochondrial disease group.

The Drop TOI varies considerably within children with mitochondrial disease and neurogenetic disease. This variation within groups is displayed in the bean plot. (Figure 4.5) Group 1 was compared to group 0 and group 2. This presents the problem of multiple comparisons. This is a small risk as there are only two comparisons. To account for this, I have included a Bonferroni correction. Accordingly, the p value that would be needed to show significance would be 0.025 (0.05/2). Both comparisons have non-significant p-values.

Figure 4.5: Comparison of Drop TOI Between Groups

Bean plots of delta tissue oxygen index (Drop TOI). The plot shows the delta tissue oxygen index (Drop TOI) with 95% CI. The subjects were classified into 3 groups healthy children (0), children with neurogenetic disease (1) and children with mitochondrial disease (2). p-value (t-test) when comparing group 0 to 1 was 0.22 while that for group 1 to 2 was 0.84. The thick black horizontal line within the yellow bean plot is the median. The red horizontal lines within the plot represent individual patient values. The width of the bean plot is proportional to the number of observations at that value.



Testing of alternative hypothesis 1

Fagan nomogram was employed to compare children with mitochondrial disease and neurogenetic disease.

Table 4.8: Comparison of children with mitochondrial disease to neurogenetic disease

Test	**Mitochondrial disease present**	**Neurogenetic disease present**	**Total**
NIRS VOT Positive	19	8	27
NIRS VOT Negative	24	11	35
Total	43	19	62

The Fisher exact test statistic was 0.98. This showed that NIRS VOT is not able to differentiate between children with mitochondrial disease and those with other causes of neurogenetic disease. The clinical utility of NIRS VOT is expected to be in a setting with varying prior probability. Hence, Fagan nomogram was employed to test post-NIRS VOT probabilities.

Testing of alternative hypothesis 2

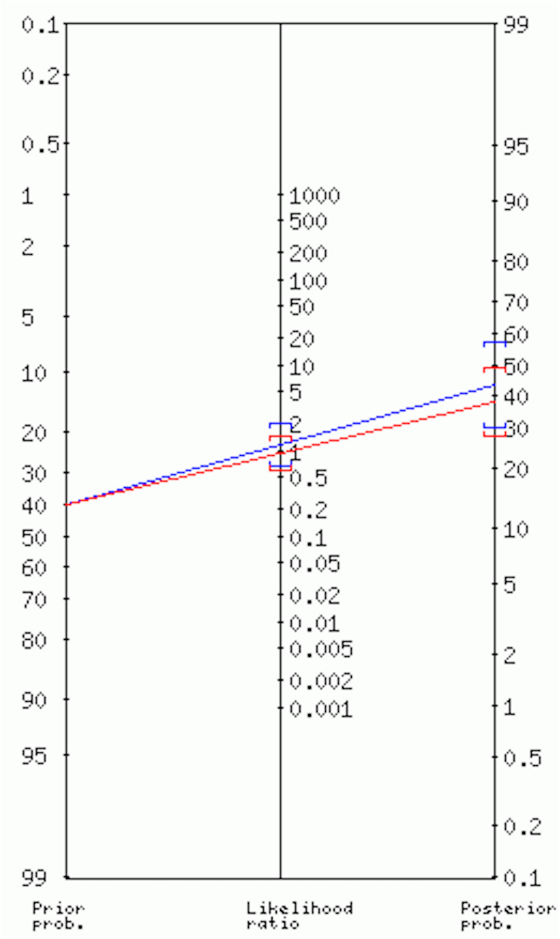
Table 4.9: Fagan nomogram posterior probabilities

Test	**Positive likelihood ratio**		**Posterior probability**		**Description**
Positive test	1.05	(95%CI:0.60-1.83)	41%	(95%CI:29-55%)	$\sim$ 1 in 2.4 children with positive test have the disease
Test	**Negative likelihood ratio**		**Posterior probability**		**Description**
Negative test	0.96	(95%CI:0.60-1.53)	39%	(95%CI:29-50%)	$\sim$ 1 in 1.6 children with negative test are well

Both positive and negative posterior probabilities are similar. This suggests that NIRS VOT is not helpful to differentiate between mitochondrial disease and other causes of neurogenetic disease.

Figure 4.6: Change in Probability of Disease After NIRS VOT Comparing Mitochondrial Disease and Neurogenetic Disease

The blue line represents the change in posterior probability following a positive NIRS VOT. The red line shows the change in the posterior probability after the NIRS VOT is negative.



In summary, the NIRS VOT derived measure of oxygen consumption (Drop TOI) is not able to differentiate between mitochondrial disease and other causes of neurogenetic disease.

Although not clinically relevant, for the purposes of my thesis I explored if NIRS

VOT is able to differentiate between children with suspected mitochondrial disease and healthy children. Near infrared spectroscopy with vascular occlusion test (NIRS VOT) was able to exclude mitochondrial disease in healthy children with a high degree of certainty (positive LR - 5.74, 95%CI:0.85-39, negative LR - 0.60, 95%CI:0.44-0.82). This suggests that the technique is able to measure oxygen consumption from the forearm muscle. This is particularly relevant for the other studies discussed in chapter 5 (Young Everest Study 2) and chapter 6 (children with critical illness).

4.5 Conclusions

This project suggests that NIRS VOT is unable to differentiate mitochondrial disease children from controls. The median Drop TOI in mitochondrial disease children is 14 compared to 17.6 in the controls. These findings are in contrast to a recent adult study comparing mitochondrial myopathy (MM) patients to healthy controls. [248] However, a comparison to healthy controls is not useful in clinical practice. The Fagan nomogram result shows that when the prior probability is high NIRS VOT is not a clinically useful tool.

The children with neurogenetic abnormality may not be the 'ideal' control population as they display a pattern of Drop TOI suggestive of secondary mitochondrial dysfunction. The variability in baseline TOI and Drop TOI observed in the neu-

rogenetic disease group is interesting and not previously reported. The utility of NIRS VOT as a diagnostic tool in this population needs further investigation.

Mitochondrial disease children might have a degree of adaption to tissue hypoxia. This may be seen as the absence of difference in NIRS VOT between this group and controls. If these tests were performed under conditions of higher oxidative stress (eg. pre and post exercise), a difference might become apparent.

Mitochondrial disease is a heterogeneous group of disorders with varied oxygen consumption. Taivassalo has addressed this variation in adult patients with MM. They performed cardiopulmonary exercise testing in 40 patients with MM and compared the oxidative capacity to 32 healthy sedentary controls. Unsurprisingly, the oxygen uptake (VO_2) was different between the groups (MM patients had lower VO_2). The VO_2 ranged from 6 to 47 ml/kg/min in MM patients. They also noted a relative increase in cardiac output in MM patients during exercise compared to controls. Furthermore, they observed a differentiation between the groups at VO_2max. The range of VO_2 is similar to the widespread of the Drop TOI noted in the current study in children with mitochondrial disease. [249]

The variability in baseline TOI noted in my study is not surprising. Baseline TOI is a marker of the resting oxygen consumption from forearm muscle. There are two main reasons for this variability. The first is that oxygen consumption at rest is inversely proportional to the mitochondrial dysfunction. [228] [250] Therefore, when

a patient has a severe disease their oxygen consumption will be lower compared to a patient who has a milder form of the disease. The second reason is disease penetrance and mitochondrial heterogeneity. Within the overall mitochondrial disease group, some diseases affect muscles while others spare muscle function. [226] In addition, even when muscle mitochondrial function is impaired there seems to be a threshold effect for this to translate to clinical signs. By extrapolation, this threshold may present as normal baseline TOI in a patient with impaired muscle oxygen consumption.

The highest TOI was statistically different between mitochondrial disease group and both controls and neurogenetic disease group. There might be two explanations for this:

1. The post occlusive reactive hyperaemia is lower in mitochondria disease children. There is no documented literature on this physiology. However, with mitochondrial dysfunction, there may be an inherent vasomotor dysfunction.

2. There is an inability to further increase recruitment of capillaries in the mitochondrial disease children in the normal state. This failure might be due to impaired miR-210 (micro RNA) expression. [251] Therefore, when the arterial occlusion is released there is a relatively lower vasomotor response.

As noted above, the cardiovascular response to NIRS VOT is dynamic in nature. Consequently, the single point measure of cardiac output in this study is per-

haps inadequate. Just the forearm occlusion during NIRS VOT might change the cardiac index. I wanted to exclude any major change in oxygen delivery by a continuous measure of the cardiac index. If this project were to be repeated, ideally a continuous non-invasive measurement of cardiac output should be employed whilst conducting the NIRS VOT. Unfortunately, no such validated non-invasive technique is currently available.

During formal cardiopulmonary exercise testing, VO_2max is achieved when further work does not increase oxygen uptake (plateau in oxygen uptake). [252] It is a measure of aerobic fitness. [253] The NIRS VOT with a 3-minute occlusion protocol does not achieve VO_2max. A longer occlusion protocol might result in achieving this limit. As noted earlier, Taivassalo et al observed a difference between MM patients and healthy controls noted a differentiation in at VO_2max. Similarly, the neurogenetic abnormality group might show different oxygen consumption at VO_2max.

One major presumption of the NIRS VOT is that the near infra-red light penetrates to a depth appropriate to interrogate the forearm muscle. The circumference of the forearm, the amount of subcutaneous fat and the muscle mass are all relevant variables that impact the measurement. Unfortunately, these were not accounted for in this project and limit the confidence in the interpretation of results.

The thickness of adipose tissue impairs the measurement of oxygen consumption. [254] This is not as relevant in children as in the adult population. Consequently, for my study I have considered it to be a systematic error and not explored this further.

Some of the children with mitochondrial disease suffer from significant dystonia. The Drop TOI will be artificially high if the child flexes their forearm during VOT. The ideal would be to perform the test only in children without dystonia. This is not feasible or pragmatic because many children with mitochondrial disease have dystonia, particularly those with Leigh syndrome, the most common presentation of mitochondrial disease in early childhood.

The children in this study had a wide age range. Furthermore, no study so far has investigated the TOI with increasing age. This may be a relevant parameter. However, my results do not show a statistically significnant relationship.

One of the potential future studies in this population might be to perform NIRS VOT under conditions of higher oxidative stress (eg pre and post exercise). This might display a reduced dro TOI. The increasing availability of whole exome and whole genome next generation sequencing (NGS) has led to a reduction in the number of muscle biopsies being performed for diagnosis of mitochondrial disease. However, NGS is time consuming. NIRS VOT may have a role whilst awaiting NGS results. NIRS VOT does not give any information about the biochemistry

or genetic analysis. It could still be an adjunct in the diagnostic pathway.

Chapter 5

Oxygen Consumption - Oxygen Delivery Relationship at Altitude: Young Everest Study 2

In chapter 3, NIRS VOT was shown as a potential bedside, non-invasive technique that could estimate oxygen consumption from the forearm muscle. This chapter explores the effects of hypoxia on the oxygen delivery-consumption relationship in healthy children. As a model of hypoxia, exposure to moderately high altitude was investigated as part of the Young Everest Study 2. All the tests were performed both at sea level and at Namche Bazaar (3525 metres).

This study was conducted to test the following hypotheses:

1) Children will have a similar cardiorespiratory response to adults when exposed to hypobaric hypoxic condtions

2) It is safe for children to trek to moderately high altitude (3525 metres) and

3) There will not be a change in oxygen consumption during the short time period (4 days) of exposure to hypobaric hypoxic conditions.

The first hypothesis was tested in the initial part of the results. In this section, cardiovascular changes at moderately high altitude are discussed - especially changes in peripheral oxygen saturations (SpO_2), heart rate (HR), respiratory rate (RR) and cardiac index (CI). A 3-minute step test was incorporated to measure post-exercise alterations in SpO_2, HR and RR. These parameters enabled a non-invasive measure of oxygen delivery and a comparison of adaptive responses noted in adult studies.

The second hypothesis was tested by the results of the cardio-respiratory responses and cerebral near infrared spectroscopy (NIRS). Cerebral NIRS (forehead) was measured at both the altitudes as a simulation of static organ perfusion and oxygen consumption.

The third hypothesis was tested by employing near infrared spectroscopy with a vascular occlusion test (NIRS VOT). This estimated dynamic forearm muscle oxygen consumption. The method of NIRS VOT was detailed in Chapter 2 (section

2.1).

The next chapter (chapter 6) compares these results to children admitted with critical illness to the paediatric intensive care unit.

I will start with a discussion on high altitude medicine research.

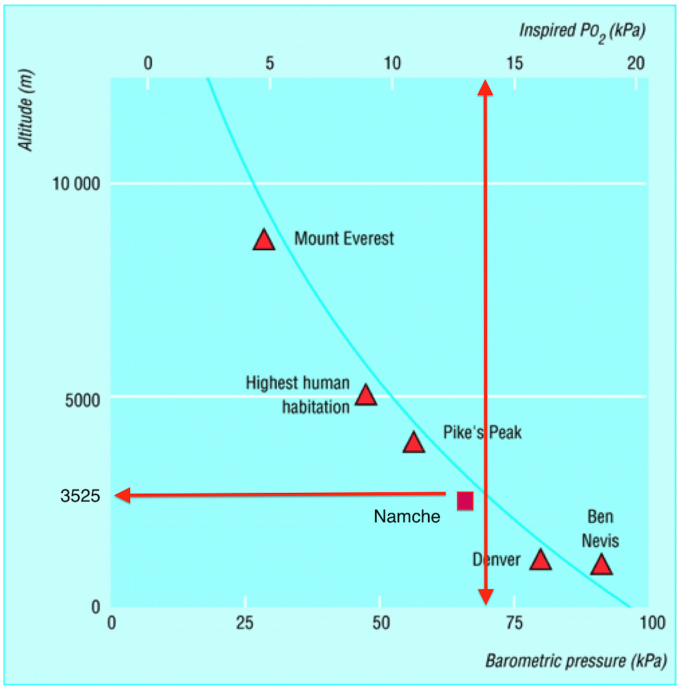
5.1 Introduction

5.1.1 High Altitude Medicine

High altitude medicine and the effect of hypoxic conditions has interested mountaineers and physicians for centuries. [2] It was known for centuries that the partial pressure of oxygen dropped with increasing altitude.(Figure 5.1) [255] Bert first published evidence linking altitude sickness and low partial pressure of oxygen. [256] Most humans do not show signs / symptoms of altitude sickness until about 3000 meters. There seems to be an altitude theeshold above which the symptoms occur and incrementally worsen. Therefore, the relationship between the partial pressure of oxygen and symptoms of altitude sickness is linear above certain altitude.

Figure 5.1: Drop in Partial Pressure of Oxygen with Altitude

Reproduced and amended from a review article by Peacock 1998. [255] The plot shows the decrease in atmospheric pressure and the partial pressure of oxygen with increase in altitude. The primary x-axis denotes the atmospheric pressure in kilopascals. The secondary x-axis shows the partial pressure of oxygen in kilopascals. The y-axis shows the various altitudes where the measurements were recorded. The red triangles denote well known high altitude destinations. The horizontal red line denotes the altitude at Namche Bazaar. The vertical red line depicts the partial pressure of inspired oxygen and the barometric pressure at Namche Bazaar.



Methodologies used in altitude research

Numerous expeditions to high altitude have studied acute physiological and evolutionary responses such as changes in the haemoglobin concentration, the ventilatory response and the shape of the chest. The main high altitude regions of

interest are the Ethiopian highlands, the Andes and the Tibetan plateau. The Ethiopian highlands are at an altitude of 3500 metres, the Andes are on average 4000 metres high while the Tibetan plateau ranges around 4000 metres above sea level. The Ethiopians have been living in the region for about 5000 years, Andeans for 11000 years and Tibetans in the Everest region for 20000 years. These populations exhibit different physiological adaptations to altitude. Andeans increase oxygen delivery by increasing their haemoglobin concentration whilst the Tibetans achieve this by an increase in minute ventilation and vasodilation (secondary to endogenous nitric oxide). The Ethiopians seem to have an adaptive physiology which is a combination of the above two mechanisms. This variability in the physiological responses can be explained by acclimatisation and natural selection. [257]

The formats employed to investigate the evolutionary response to altitude are:

1. Single population with multiple stresses (such as Quechua population studies). For example, Quechua studies showed that children living in southern Peru at an altitude of 4000-5500 metres had a slower and prolonged growth compared to children at sea level in the United States and Peruvian lowlands. However, they had relatively larger chest circumferences and forced vital capacities. [258]

2. Multiple populations with single stress - such as comparing Ethiopians to Andeans and Sherpas i.e comparing highlanders living at similar altitudes.

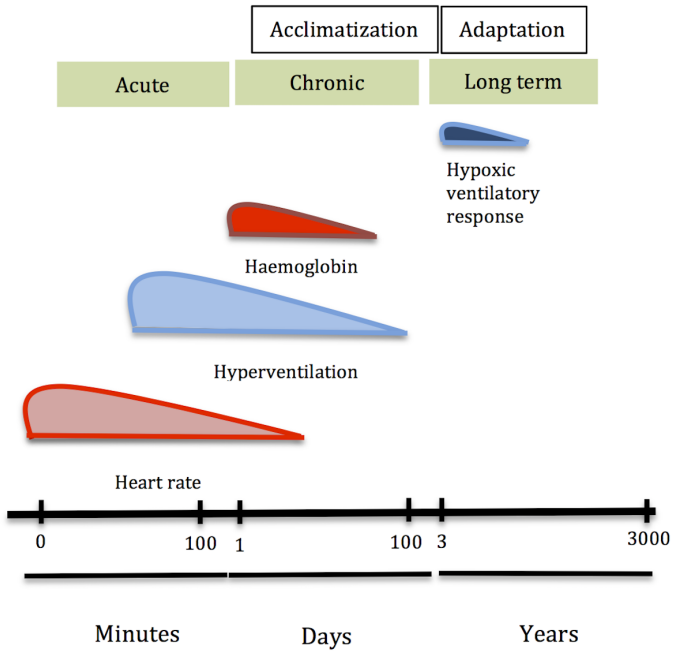
3. Quantitative genetics approach. [257]

Another method of investigating a single population would be the migrant study design. The Muza study observed that resting and exercise oxygen saturations at high altitude dropped initially on day 1 followed by a marginal stabilisation. A reverse pattern was noted with the exercise heart rate. [259] This study is of particular interest as the Young Everest Study 2 explored these parameters in healthy children.

Over a longer period the above-mentioned responses enable the understanding of human evolutionary responses. For example, commonly measured cardio-respiratory parameters such as heart rate and respiratory rate change in the acute phase whilst the adaptive responses such as hypoxic ventilatory response take years to establish. [2] (Figure 1.2 and Figure 1.3).

Figure 5.2: Acclimatization over a Long Period of Time

Adapted from the book by West, Schoene and Milledge 2007. [2] The acclimatisation is separated into acute (in minutes) and days (up to 100 days). Most of the acclimatization responses revert to baseline values in a short span of time. Heart rate, ventilation and haemoglobin would be expected to revert to baseline values by 100 days. The long-term adaptations are trans-generational.



The figures 5.5 and 5.6 summarise the physiological changes with hypobaric hypoxia in healthy adult volunteers. The muscle fibre cross-sectional area, skeletal muscle mass and muscle mitochondrial volume density are also reduced. [260] [261]

Figure 5.3: Changes in the Cardiovascular Responses at High Altitude After a Period of Acclimatization

Created from text in the article by Martin et al 2008. [261] The parameters on the left hand side increase during acclimatisation while the ones on the right hand side decrease.

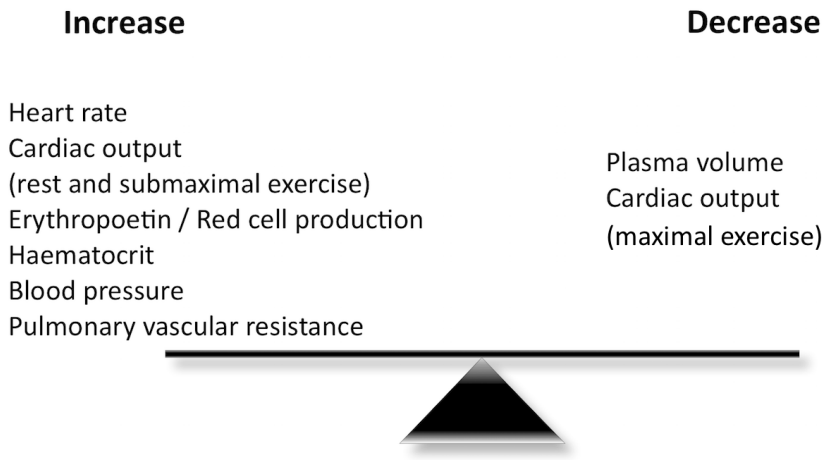
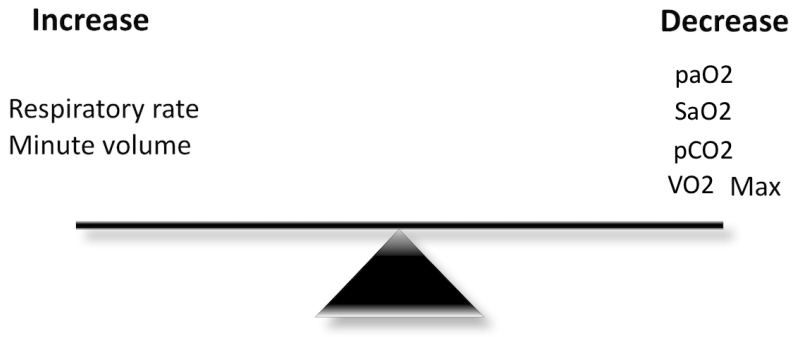


Figure 5.4: Changes in the Respiratory System Responses at High Altitude After a Period of Acclimatization

Created from text in the article by Martin et al 2008. [261] The parameters on the left hand side increase during acclimatisation while the ones on the right hand side decrease.PaO_2 - Partial pressure of oxygen in arterial blood, SaO_2 - Oxygen saturation of arterial blood, PCO_2 - Partial pressure of carbon dioxide, VO_2 - Oxygen consumption



All the above-mentioned responses are with an eventual aim to optimise the oxygen delivery-consumption relationship. Lets now discuss the oxygen cascade.

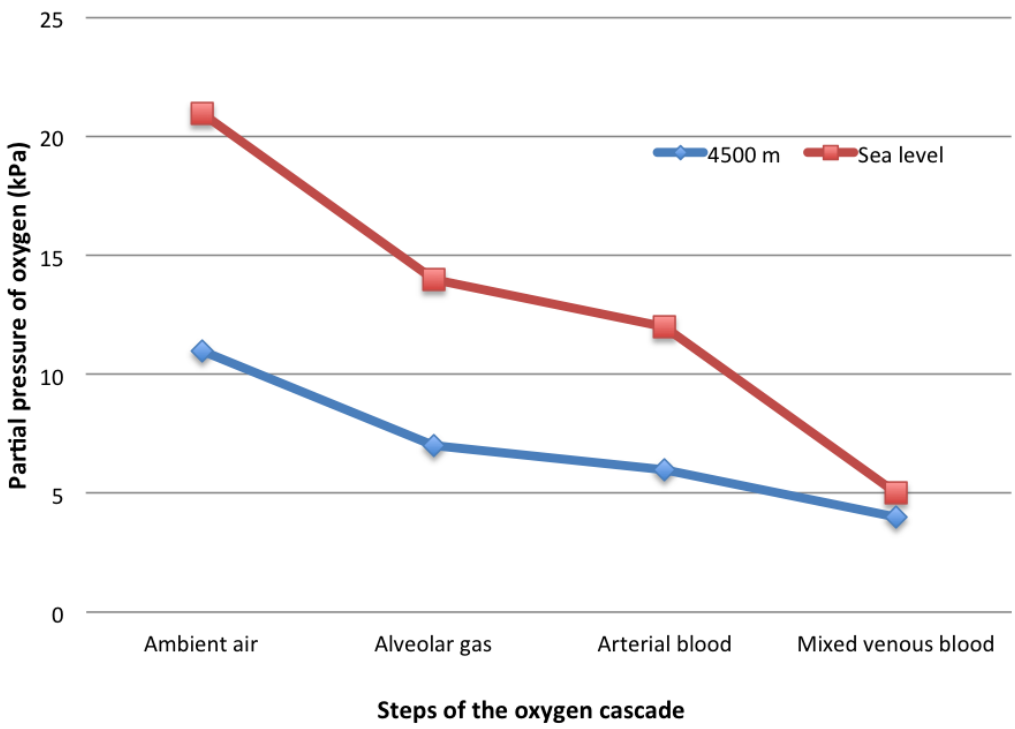
5.1.2 Oxygen Cascade at High Altitude

The oxygen cascade in a healthy human being at sea level (discussed in chapter 1.4) shows a sequential drop in the partial pressure of oxygen (PO_2) from ambient air through to the tissues. This decrease in PO_2 is more gradual at high altitude in adapted individuals. [262] [263] Consequently, although the PO_2 in ambient air is significantly lower at 4500 metres, the levels achieved in the mixed venous blood are nearly the same. Figure 5.1 depicts this difference in the rate of drop. Several mechanisms may be at play here. In the lung, diffusion of oxygen from the inspired air into arterial blood may be more efficient at high altitude. In the presence of pulmonary oedema, this is unlikely. In the tissues, there may be a relative decrease in oxygen extraction from the arterial blood, perhaps due to a more efficient utilisation of the available oxygen. Another mechanism in action at high altitude is the relative drop in PCO_2. In the healthy adult volunteer, there is an inverse relationship between PaO_2 and PCO_2 at high altitude. Due to hypoxic ventilatory response and hypercapnic ventilatory response the minute ventilation increases and results in the drop in PCO_2. Consequently, increasing the PaO_2, the arterial oxygen content and the oxygen delivery. An understanding of the dif-

ference in slope of the oxygen cascade at high altitude under hypoxic conditions could potentially be translated to the hypoxic patient on the intensive care unit.

Figure 5.5: Comparison of Oxygen Cascade Between Altitudes

Adapted from Torrance 1970. [263] The plot shows the decrease in the partial pressure of oxygen down the oxygen cascade. The x-axis denotes points of measurement - the oxygen molecule travels from ambient air through the alveolar gas, arterial blood to the tissues. Mixed venous blood denotes the value after extraction of oxygen in the tissues. The y-axis shows the partial pressure of oxygen in kilopascals.



5.1.3 Relevance of Studying the Effects of Hypobaric Hypoxia to the Hypoxic Patient on the Intensive Care Unit

Hypoxic patients in intensive care are in a normobaric environment. Although most studies at high altitude investigate effects of hypobaric hypoxia, some crossover trials have compared hypobaric hypoxia and normobaric hypoxia. A review by Coppel found that most of the physiological parameters compared were similar between the groups. Several of the studies reported a difference in minute ventilation and exhaled nitric oxide (NO) (total - 13 cross-over trials). These differences have to be considered with caution. Not only did the studies include a small number of participants, but also did not account for confounders such as duration of exposure. Nonetheless, investigating response to hypobaric hypoxia may help understand the clinical status in normobaric hypoxia patients. [264]

My YES2 expedition was conducted under the auspices of the Xtreme Everest adult study. I will enumerate the salient studies conducted by the adult team below. I will discuss studies investigating macrocirculation, microcirculation, metabolic pathways, mitochondrial number and finally genetic polymorphisms.

5.1.4 Results from the Xtreme Everest Study

I will discuss studies investigating macrocirculation, microcirculation, metabolic pathways, mitochondrial number and finally genetic polymorphisms.

Maximal Oxygen Consumption

Formal cardiopulmonary exercise tests were performed in healthy volunteers at sea level and Everest base camp by the Xtreme Everest team. They noted a significant drop in maximal oxygen consumption from 43.8±5.4 mls/kg/min at sea level to 30.2±3.8 mls/kg/min in Everest Base Camp. [176] The relationship between oxygen delivery and consumption is yet to be reported. However, preliminary results seem to suggest a decrease in consumption that is not correlated with the delivery. This might be a marker of alterations in mitochondrial function. The basic tenet of goal-directed therapy (GDT) is to optimise oxygen delivery to match oxygen consumption. These results would be crucial and might assist with targets for GDT in the intensive care patient.

Skeletal Muscle Oxygen Consumption

Near infrared spectroscopy with a vascular occlusion test was employed by Martin et al. They observed that baseline tissue oxygen measurement was reduced at altitude compared to sea level. In addition, the rate of recovery post-arterial

occlusion was significantly lower at high altitude. Of note, they did not find a difference in the rate of desaturation after arterial occlusion. [265] If the rate of desaturation and the lowest TOI were unchanged during adaptation despite hypoxic conditions, then the only explanation would be that body has become more efficient at extracting oxygen. The hypoxic intensive care patient demonstrates varying oxygen consumption profiles depending on the aetiology. For example, patients with a cytotoxic tissue injury might utilise less oxygen whilst those in a hyperdynamic warm shock state may need more oxygen. Hence it would be difficult to extrapolate the Martin study findings without further research.

Alterations to Microcirculation

Martin et al investigated peripheral microcirculation using a sidestream darkfield camera. In 24 healthy volunteers, they observed that microvascular capillary flow reduced, while vessel density increased with ascent to 5300 meters. [266] In normal circumstances only a small proportion of the capillary bed is perfused. The 'unperfused' vessels are perhaps recruited when there is a need for increased perfusion. [267] The increased capillary density noted in hypobaric hypoxic conditions suggests a reduction in diffusion distance. This might account for a net augmentation of perfusion despite a drop in flow. This might eventually lead to better oxygen diffusion from slowly moving red blood cells from numerous capillaries.

Nitric oxide may be a potent mediator in the recruitment of capillaries. In addition to recruitment, neovascularisation may occur under hypoxic conditions by the production of vascular endothelial growth factor by stimulating the hypoxia inducible factor system. [268] Despite these theories, it is still unclear if these microcirculatory changes are adaptive or maladaptive.

Decreased microcirculatory flow is associated with poor outcome in adult patients with septic shock. [269] If we explore the adaptive mechanisms in microcirculation at altitude, this might then be translated to the directed management of the septic intensive care patient.

Changes to Metabolic Pathways

Roberts et al investigated responses to exposure to altitude in 6 beta-blocked adults and 5 healthy controls. They measured free fatty acid (FFA) and fatty acid (FA) levels (at rest and after exercise) at sea level and after chronic exposure to an altitude of 4300 meters. After acclimatization, resting FA consumption decreased to zero. Following exercise, FA consumption increased but did not reach sea level values (relative decrease). In addition, glucose uptake increased compared to sea level. Therefore, exercise at altitude increases glucose uptake and decreases fat utilisation. Beta-blockade seemed to potentiate these changes. [270] The increase

in FFA levels might be due to lipolysis secondary to increased sympathetic neural activity. The enhanced glucose uptake is perhaps secondary to an increase in basal metabolic rate. [271] With neutral energy and nitrogen balance in the Roberts study, there is a suggestion that body transfers to a solely carbohydrate based fuel consumption. This is an interesting result for the intensivist especially as the ideal nutritional supplementation of the catabolic patient in intensive is unclear.

Cooper et al investigated glucose utilisation during exercise in healthy volunteers subject to hypoxic (FiO_2 - 0.15) and hyperoxic (FiO_2 - 0.8) environments. They noted a significant increase in glucose uptake under hypoxic compared to hyperoxic conditions. This finding is similar to the Roberts study results. [272] I did not investigate these metabolic pathways as part of the YES2 study. However, they may help explain my results from the investigation of oxygen consumption studies on the paediatric intensive care population (Chapter 6).

Changes to Mitochondrial Density

With decrease in oxygen supply under hypoxic conditions (at high altitude), the body needs to be more efficient and produce relatively similar amounts of ATP for the available oxygen (lower). In order to reduce oxygen consumption, the body

responds by decreasing the mitochondrial density. The exact mechanism by which this reduction is achieved is unclear. Mitophagy might be one potential process. Levitt et al performed muscle biopsies in 18 subjects in London and at Everest Base Camp (5400 meters). They observed a 21% drop in mitochondrial volume in Everest climbers and 14% drop in Base Camp climbers compared to sea level values. Furthermore, the value of transcriptional protein peroxisome proliferator-activated receptor coactivator-1 α (PGC-1 α) decreased by 35% suggesting decreased mitochondrial biosynthesis. Therefore, there is a reduction in number of mitochondria with a drop in production. [176] The explanation for this adaptive response could be that mitochondria become more efficient in producing ATP. However, to prove this we need to show that there is no deficit in energy production.

Interventions to increase oxygen delivery could potentially be withheld if this adaptive mechanism was to occur in the hypoxic intensive care patient, without compromising recovery from critical illness.

Unique Genetic Polymorphisms

The variations in inter-individual adaptations, the propensities to altitude sickness and the level of neurological function at high altitude are known. [273] In addi-

tion, numerous genotypes have been explored in this context. [274] For example, an Angiotensin Converting Enzyme (ACE) polymorphism has been recently studied. Angiotensin Converting Enzyme limits vasodilation and causes vasoconstriction through the Renin-Angiotensin system. It has two alleles - insertion (I) and deletion (D). The ACE insertion allele is associated with lower circulating ACE levels and is more commonly found in elite endurance athletes. [275] Thompson et al conducted a prospective study to investigate if ACE I allele is overrepresented in those who successfully climbed over 8000 meters. They observed a statistically significant association between presence of I-allele and success. [276]

A few mechanisms might explain the above findings. First, the I-allele group seem to have an enhanced ventilatory response. Second, the I-allele may be protective against Hypoxic Pulmonary Vasoconstriction Response. Finally, it I-allele groups might have more diuresis reducing the risk of pulmonary edema. [277] These mechanisms may also confer a protective benefit to the hypoxic intensive care patient.

The studies mentioned in this section and the mechanisms explored, support the relevance of research in a hypobaric hypoxic environment in healthy volunteers. Critical illness is characterised by an imbalance between oxygen delivery and consumption, causing a net oxygen deficit. Therefore, by understanding how

adaptive mechanisms function in those exposed to hypobaric hypoxia (to prevent oxygen deficit), we can potentially develop therapeutic targets in critically ill patients to enhance adaptation. In summary, to aid with optimising oxygen delivery-consumption relationship, within the cell, mitochondrial density is reduced, the electron transport chain is downregulated and oxidative phosphorylation becomes more efficient (more ATP production for the same amount of oxygen consumed). [186] I will now briefly discuss altitude research in children and the use of near infrared spectroscopy.

5.1.5 Altitude Research in Children

Children may have a different response to high altitude exposure compared to adults secondary to the maturing sympathetic and chemoreceptor reflex systems. They have less nocturnal periodic breathing at high altitude. [278] Nocturnal periodic breathing is a commonly noted phenomenon in healthy humans at altitude. The pattern has periods of hyperventilation and central hypoventilation. It may cause increased behavioural awakenings at altitude. [279] In addition, the prevalence of acute mountain sickness in pre-pubertal children and adolescents may be less. [280] Yaron investigated the haemodynamic response during acute exposure to high altitude in 24 children. Children between 3 months and 36 months of age were accompanied to an altitude of 3109 metres. They demonstrated an increase

in respiratory rate and a decrease in oxygen saturation at higher altitude. These changes are similar to adults. [281] Kriemler reported similar results. [282] However, the effect of sub-acute exposure remained unanswered. The Young Everest Study (YES) explored this area for the first time.

The YES team trekked to an altitude of 3525 metres over a 4-day period. The cardio-respiratory responses of 9 children between the ages 6 and 13 years were measured. An increase in heart rate and drop in oxygen saturation was observed. The respiratory rate increased marginally and seemed to drop towards sea level values with longer duration at high altitude. Furthermore the oxygen saturation increased by the end of their stay at high altitude. This is perhaps due to an increase in oxygen delivery. The YES study was not designed to measure the parameters needed to calculate oxygen delivery (DO_2) and oxygen consumption (VO_2). [283] The Young Everest Study 2 (YES2) set out to answer some of these questions.

5.1.6 Near Infrared Spectroscopy at High Altitude

Cerebral near infrared spectroscopy (NIRS) has been employed both in adults and children visiting high altitude. Yaron studied cerebral NIRS in his study. He found an age-related drop in tissue oxygen saturations with an acute ascent

to high altitude (3109 metres). [281] Cerebral NIRS was performed in children needing air ambulance transfer. They found a statistically significant drop in cerebral oxygen saturation in children who had a cruising altitude of greater than 5000 metres and in mechanically ventilated children. [284] I have utilised this technique to objectively estimate static oxygen saturation of the organ. This is relevant especially when considered in the context of changing cardiac index and the whole body hypoxia (lower peripheral oxygen saturation).

The second technique that I have utilised is the near infrared spectroscopy with vascular occlusion test (NIRS VOT). Martin et al performed NIRS VOT at sea level and at Everest base camp (5600 metres) in 12 healthy adult volunteers. This study was described earlier under Xtreme everest studies. I performed the same study in healthy children to evaluate the dynamic oxygen consumption from forearm muscle.

5.2 Aims

The aims of the Young Everest Study 2 (YES2) were two-fold:

1. To describe the change in the cardiorespiratory parameters in children with a gradual ascent to a hypobaric hypoxic environment - especially change in peripheral oxygen saturations, heart rate, respiratory rate and cardiac index.

2. To investigate if there is a change in the pattern of non-invasive measures of

DO_2 and VO_2 under hypoxic conditions in healthy children - Change in cerebral near infrared spectroscopy, near infrared spectroscopy vascular occlusion test (NIRS VOT) and cardiopulmonary exercise test (CPET).

5.3 Methods

The study was conducted as a paediatric subgroup under the auspices of the Xtreme Everest 2 study. Children were recruited from healthy volunteers. A total of 12 children participated in the study. The baseline sea level testing (SLT) was performed in London (altitude - 30 metres) over a two-day period. The sea level testing involved measurement of cardio-respiratory parameters (pre and post-step test), cardiac index (measured by USCOM - described in section 2.2) at rest, cerebral near infrared spectroscopy and near infrared spectroscopy vascular occlusion test (NIRS VOT - described in section 2.1) at rest. All the tests were performed in a laboratory with a set room temperature of 20 °C.

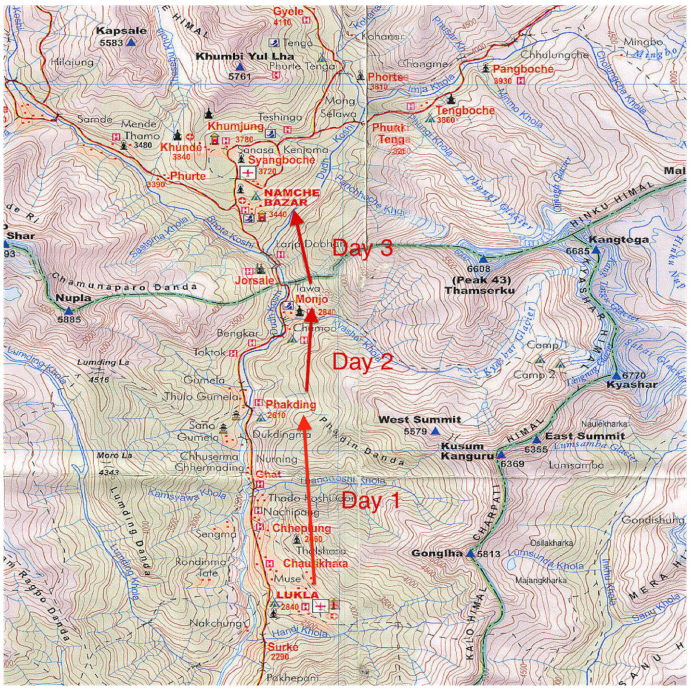
The sea level testing was followed (2 months later) by an expedition to the Everest region in Nepal. The children and their families were accompanied to Nepal by 6 paediatric investigators. The expedition lasted two weeks (27^{th} March - 12^{th} April 2013).

The expedition started with a flight from London to Kathmandu (1400 metres). In Kathmandu, the heart rate (HR), respiratory rate (RR) and oxygen saturations

(SpO_2) pre and post a 3-minute step test were measured. The cardiac index (CI) at rest was also measured. After a day's rest, the team took a short flight from Kathmandu to Lukla (2860 metres). The team reached Namche Bazaar on day 4 following a 3-day gradual ascent (overnight stopovers at Phakding (2610 metres) and Monjo (2835 metres)). Figure 4.2 depicts the trek profile (amended from image downloaded from Google images). The sea level tests were repeated over two days at Namche Bazaar.

Figure 5.6: Trek Profile of the Young Everest Study 2 Team

Amended from image downloaded from Google images. The team travelled from London to Kathmandu. After an overnight stay, we flew to Lukla. From Lukla, we reached Namche Bazaar over a three-day period with 2 overnight stops at Phakding and Monjo.



5.4 Statistical Analysis

The heart rate, respiratory rate, peripheral oxygen saturation (SpO_2) and cardiac index were compared between altitudes. The analysis was performed using the repeated measures of ANOVA. Published normal ranges of heart rate in children were utilised to calculate the heart rate Z scores using the LMS method. [285] [286] Cerebral NIRS measurements in London and Namche Bazaar were compared with student t-test. Analysis of near infrared spectroscopy vascular occlusion test (NIRS VOT) employed the following techniques:

1. Multilevel modelling by considering measurements at different altitudes as nested values within an individual child.

2. Functional data analysis by using the full 13-minute curve of NIRS VOT to compare groups.

5.5 Results

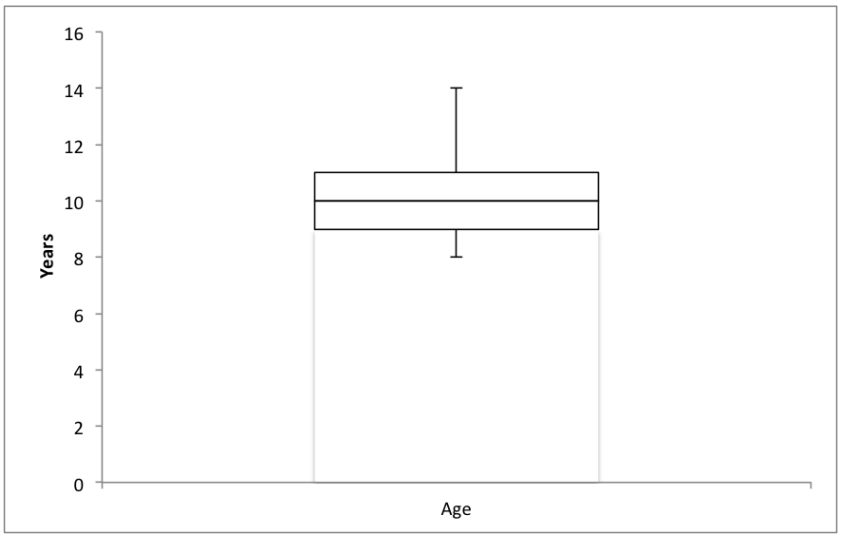
The barometric pressure is 503 mmHg at Namche Bazaar. The PiO_2 drops from 150 mmHg at sea level to 96 mmHg at Namche Bazaar.

The age range of the children was between 8 and 16 years (Figure 5.7). There were 7 boys and 5 girls in the group. Most of the children (11) were able to complete the trek to Namche Bazaar and back successfully. One child became unwell whilst

in Namche Bazaar (Salmonella typhi gastroenteritis) and was evacuated back to Kathmandu.

Figure 5.7: Age Distribution of Volunteers in the Young Everest Study 2

The children ranged from 8 years to 14 years. The horizontal line within the box denotes median value. The whiskers represent 1st and 3rd quartile values.

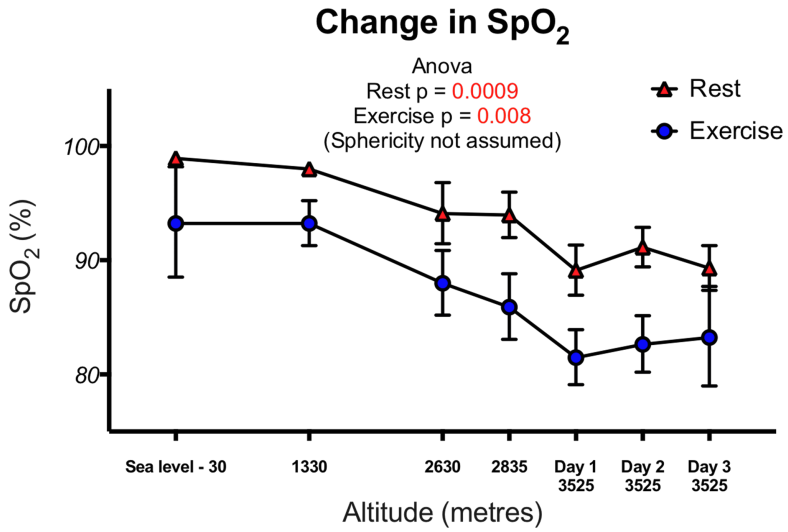


5.5.1 Peripheral Oxygen Saturation

The SpO_2 at rest and exercise dropped with increasing altitude. The SpO_2 at rest decreased from 99 ± 0.79 to 89 ± 2.54 % from London to Namche Bazaar (p=0.0009). The SpO_2 after exercise decreased from 93.5 ± 7.16 to 83.3 ± 5.06 % from London to Namche Bazaar (p=0.008). There was no impact of duration of stay on the SpO_2 at rest and after exercise. This could be the first sign of acclimatisation with an increase in arterial oxygen content. Figure 5.9 illustrates changes in resting and exercise oxygen saturation with altitude.

Figure 5.8: Change in Peripheral Oxygen Saturation with an Ascent to High Altitude

The plot depicts the decrease in oxygen saturation with an ascent to high altitude. The y-axis shows peripheral oxygen saturations. The x-axis shows the different stages in the ascent. The first measurement was at sea level. After a three-day trek the children reached Namche Bazaar at an altitude of 3525 metres. At Namche Bazaar, three days of recording were performed. The red triangles with connecting black lines depict saturations at rest. The blue circles with connecting black lines show the saturations after a 3-minute step test. The black vertical lines depict 95% confidence intervals. The p-value was calculated with repeated measures ANOVA. The p-value for saturations at rest was 0.0009 and for post-exercise was 0.008.



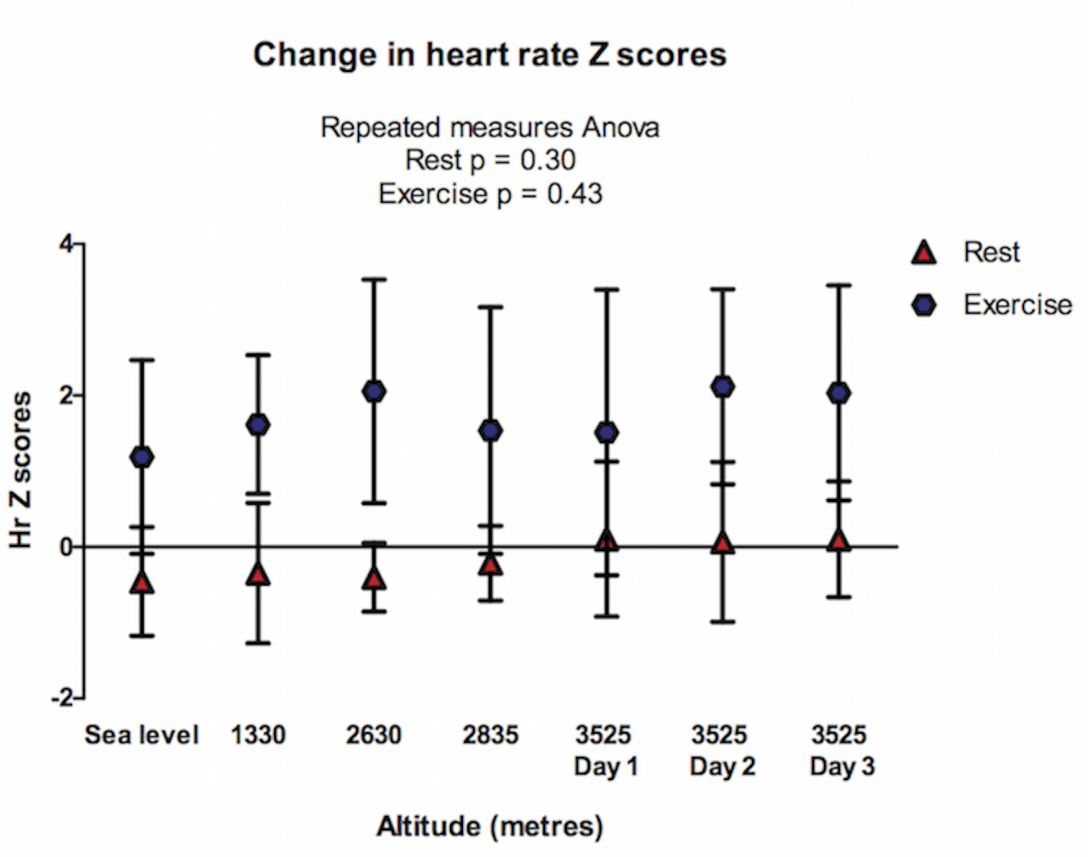
5.5.2 Heart Rate

Heart rate increased with altitude both at rest and after exercise. There was more variation in heart rate after exercise. The increase in heart rate was not statistically significant when Z-scores were used. The heart rate Z-scores at rest changed from -0.44 ± 0.70 to 0.10 ± 0.76 bpm (p=0.30) whilst the heart rate Z-scores after

exercise increased from 1.20 ± 1.30 to 2.07 ± 1.46 (Figure 5.10).

Figure 5.9: Change in Heart Rate (Z-scores) with an Ascent to High Altitude

The plot depicts the change in heart rate Z-scores with an ascent to high altitude. The y-axis shows heart rate Z-scores. The x-axis shows the different stages in the ascent. The first measurement was at sea level. After a three-day trek the children reached Namche Bazaar at an altitude of 3525 metres. At Namche Bazaar, three days of recording were performed. The red triangles with connecting black lines depict heart rate Z-scores at rest. The blue circles with connecting black lines show the heart rate Z-scores after a 3-minute step test. The black vertical lines depict 95% confidence intervals. The p-value was calculated with repeated measures ANOVA. The p-value for heart rate at rest was 0.30 and for post-exercise was 0.43.

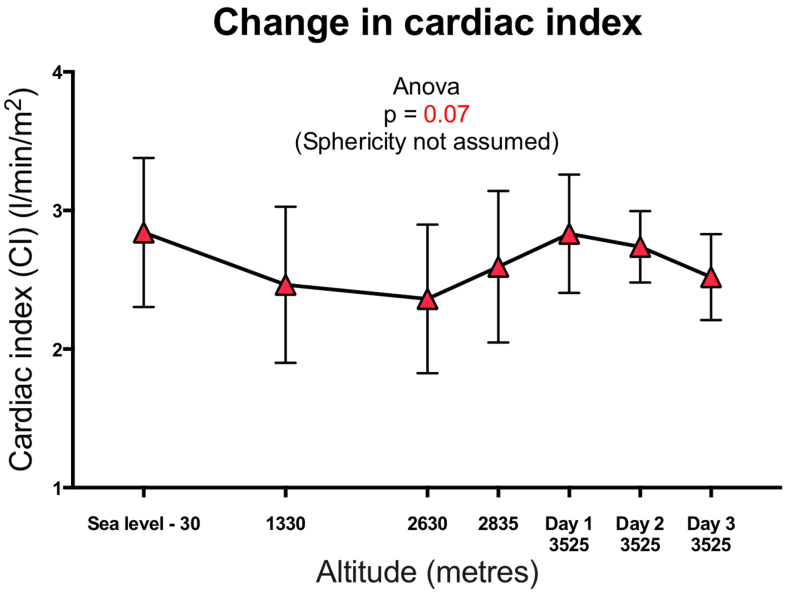


5.5.3 Cardiac Index

Cardiac index was measured at rest 3 times every morning and the average considered the final value. It did not change significantly with an ascent to high altitude. (Figure 5.11)

Figure 5.10: Change in Cardiac Index with an Ascent to High Altitude

The plot depicts the change in cardiac index with an ascent to high altitude. The y-axis shows cardiac index. The x-axis shows the different stages in the ascent. The first measurement was at sea level. After a three-day trek the children reached Namche Bazaar at an altitude of 3525 metres. At Namche Bazaar, three days of recording were performed. The red triangles with connecting black lines depict cardiac index measured at rest. This measurement was not repeated after exercise. The black vertical lines depict 95% confidence intervals. The p-value, calculated with repeated measures ANOVA, was 0.07.



5.5.4 Respiratory Rate

Respiratory rate after exercise increased consistently from sea level (22.2 ± 4.4) to Namche Bazaar (27.6 ± 10.9). This is in keeping with the expected increase in minute ventilation. Table 5.1 enumerates the change in barometric pressure and partial pressure of inspired oxygen (PiO_2). The values of barometric pressure and PiO_2 were referenced from article by Scrase et al. [283] Table 5.1 also gives values of respiratory rate (at rest and after exercise) and heart rate (at rest and after exercise). Table 1.2 gives details of the changes in SpO_2, heart rate Z-score (at rest and after exercise), cardiac index and DO_2I (oxygen delivery index) with increasing altitude from sea level to Namche Bazaar (3525 metres).

Table 5.1: Changes in vital signs with increasing altitude in healthy children

Altitude	**Sea level**	**1330 mts**	**2630 mts**	**2835 mts**	**3525 mts (day 1)**	**3525 mts (day 2)**	**3525 mts (day 3)**
Barometric pressure (mmHg)	760	652	560	548	503	503	503
PiO_2 (mmHg)	150	127	108	105	96	96	96
RR/min (rest)	19.3 ± 4.6	18.2 ± 4.0	17.6 ± 3.1	17.8 ± 4.2	17 ± 3	17.4 ± 3.5	21.3 ± 7.3
RR/min (exercise)	22.2 ± 4.4	20.9 ± 3.5	24.2 ± 3.8	23.2 ± 3.8	26.5 ± 4.7	24.3 ± 4.8	27.6 ± 10.9
HR/min (rest)	77.5 ± 13.0	80 ± 19.9	78.6 ± 8.9	82.4 ± 9.9	89 ± 20.1	88.1 ± 19.7	88.1 ± 12.8
HR/min (exercise)	111.1 ± 23.8	119.6 ± 19.5	129.4 ± 29.4	118.5 ± 30.9	118.2 ± 39.0	130.3 ± 26.2	127.8 ± 26.5

Key: RR - Respiratory rate, HR - Heart rate, PiO_2 - Partial pressure of oxygen

Table 5.2: Changes in haemodynamic parameters in healthy children with an ascent to high altitude

Measure	**Sea level**	**1330 mts**	**2630 mts**	**2835 mts**	**3525 mts (day 1)**	**3525 mts (day 2)**	**3525 mts (day 3)**	**p-value**
SpO_2 (rest)	98.9 ± 0.8	97.8 ± 0.8	93.1 ± 4.1	94.0 ± 1.9	88.2 ± 3.3	91.4 ± 2.7	89.3 ± 2.5	0.0009
SpO_2 (exercise)	93.5 ± 7.2	93.2 ± 2.5	88.7 ± 3.1	88.4 ± 3.2	83.4 ± 2.8	82.0 ± 3.7	83.3 ± 5.1	0.008
HR Z-score (rest)	-0.4 ± 0.7	-0.4 ± 1.0	-0.5 ± 0.4	-0.3 ± 0.5	-0.1 ± 1.0	-0.1 ± 1.1	0.1 ± 0.8	0.30
HR Z-score (exercise)	1.2 ± 1.3	0.8 ± 2.3	2.4 ± 1.6	1.9 ± 1.9	1.7 ± 2.1	1.9 ± 1.4	2.1 ± 1.5	0.43
CI $(L/min/m^2)$	3.0 ± 0.5	2.4 ± 0.6	2.3 ± 0.6	2.6 ± 0.6	2.7 ± 0.3	2.7 ± 0.3	42.5 ± 0.3	0.07

Legend: The values presented are *mean*±standard deviation. P-value was calculated with repeated measures ANOVA. SpO_2 - Peripheral oxygen saturation, HR - Heart rate, CI - cardiac index. SpO_2 was recorded as a percentage.

5.6 Near Infrared Spectroscopy

5.6.1 Cerebral Near Infrared Spectroscopy

The left frontal tissue oxygen index (TOI) in London was 67.7. The right frontal TOI in London was 67.7. The left frontal TOI in Kathmandu was 70.2 while the right frontal TOI was 70.3. The left frontal TOI in Namche Bazaar was 68 while the right frontal TOI was 68.1. There was no difference between London and Namche Bazaar (p- value: Left - 0.70, Right - 0.54). (Figures 5.12 and 5.13)
This is interesting as the SpO_2 values dropped at high altitude. Therefore, cerebral TOI seems to be preserved in the presence of hypoxia.

Figure 5.11: Left Frontal Cerebral Near Infrared Spectroscopy

The recording is for left frontal near infrared spectroscopy at three altitudes - London (sea level), Kathmandu (1330 metres) and Namche Bazaar (3525 metres). The y-axis depicts the tissue oxygen index value from near infrared spectroscopy. The x-axis denotes the three sites where the recordings were performed. The bean plot in yellow shows the distribution of the values. The red horizontal lines within the bean plot show individual subject values. The bold black horizontal line depicts the median value. T-test comparing values at London to Namche Bazaar was 0.70.

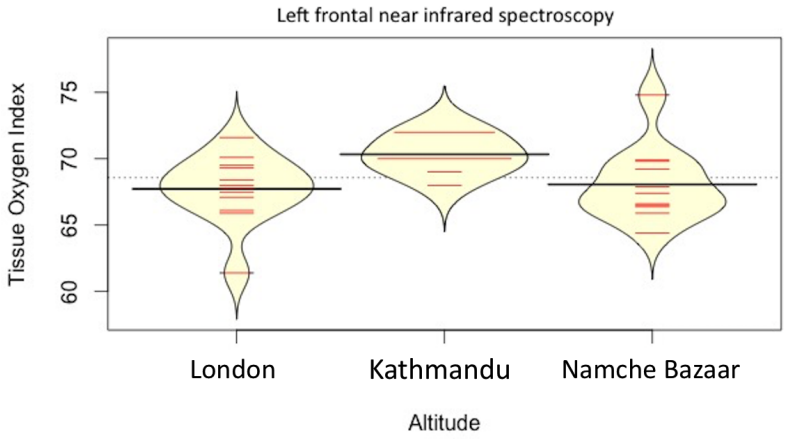
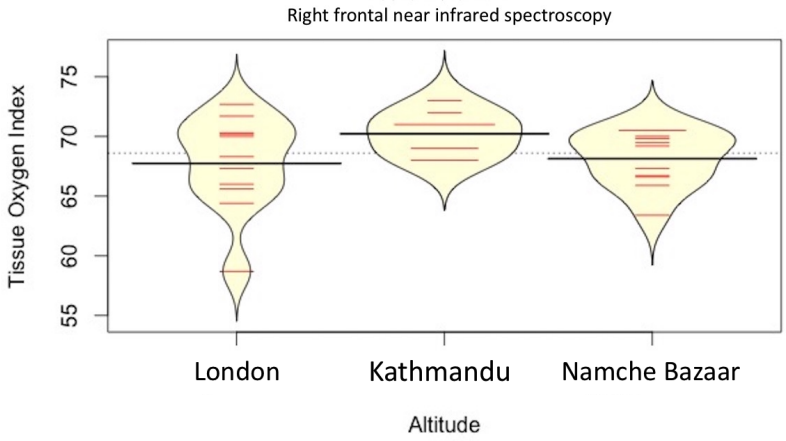


Figure 5.12: Right Frontal Cerebral Near Infrared Spectroscopy

The recording is for right frontal near infrared spectroscopy at three altitudes - London (sea level), Kathmandu (1330 metres) and Namche Bazaar (3525 metres). The y-axis depicts the tissue oxygen index value from near infrared spectroscopy. The x-axis denotes the three sites where the recordings were performed. The bean plot in yellow shows the distribution of the values. The red horizontal lines within the beanplots shows individual subject values. The bold black horizontal line depicts the median value.T-test comparing values at London to Namche Bazaar was 0.54.



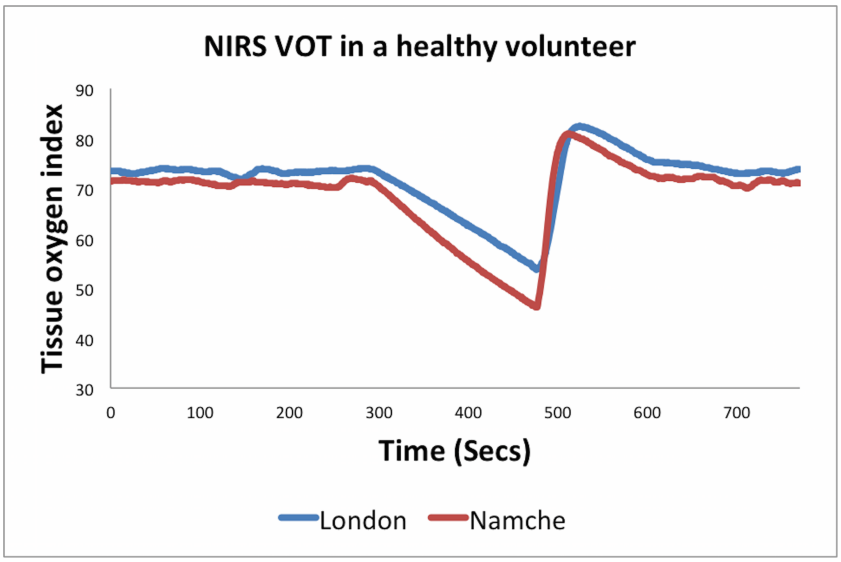
Although I am not presenting data on cerebral oxygen delivery, another member of the YES2 team measured these by using transcranial doppler. She found no correlation between the cerebral near infrared spectroscopy values and the cerebral oxygen delivery measured with transcranial doppler.

5.6.2 Variation in Oxygen Consumption - Near Infrared Spectroscopy with a Vascular Occlusion Test Results

Near infrared spectroscopy with vascular occlusion test (NIRS VOT) was used to assess the oxygen consumption both at sea level and at 3525 metres altitude. Figure 5.13 shows a recording of NIRS VOT from one of the volunteer children in London and at Namche Bazaar. The curve is made up of 4 sections - baseline, downslope, upslope and recovery. The baseline is lower at Namche Bazaar (red curve) compared to London. Also, the down slopes after the cuff inflation (at about 300 seconds) seem different. I will analyse this using two techniques: a multilevel model and functional data analysis.

Figure 5.13: An Example of Change in the NIRS VOT Curve with Altitude

The blue curve was recorded at sea level (London) and the red curve was recorded from the same child after ascent to high altitude (Namche Bazaar). Both curves comprise of 4 sections: baseline, drop, upslope and recovery.

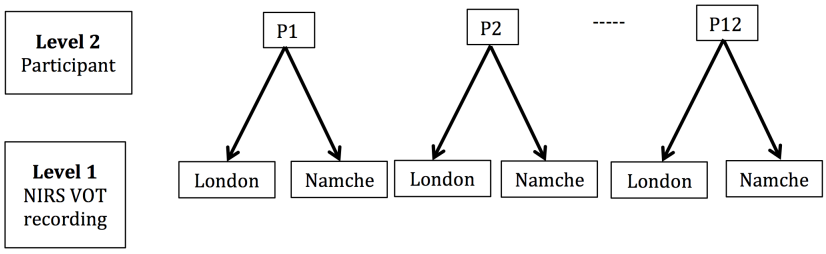


5.6.3 Multilevel Model

The NIRS VOT recordings at the two altitudes from the same participant are likely to be more similar compared to that from other participants (matched pairs). Hence, this data should be analysed in a multilevel model. In my model, the individual participant is placed at level 2 and the recordings are nested within this at level 1. (Figure 5.14)

Figure 5.14: Multilevel Model

Organisation of the levels for analysis of YES2 NIRS VOT data



In the multilevel regression model, the tissue oxygen index (TOI) was the outcome measure. The time variable of the four sections of the NIRS VOT curve was coded as baseline, downslope, upslope and recovery (for ease we will call this 'timecode'). The fixed effects co-variates were cardiac index, altitude, timecode and an interaction term between altitude and timecode. An interaction term was needed as I hypothesised that the timecode would vary between the altitude. Altitude was used as the random effects co-variate. Table 5.3 gives the beta-coefficients for fixed effects, standard errors for the coefficient and statistical significance. Due to restrictions with R software employed, this multilevel model did not report p-values. But the statistical significance can be extrapolated from the standard error values.

The coefficients for the downslope, upslope and recovery are normalised for baseline section of the NIRS VOT curve. Similarly, the interaction term (altitude and timecode) was normalised for (altitude x baseline).

Table 5.3: Multi-level model results

Co-variate	**Coefficient**	**Standard error**	**Statistically significant**
Downslope	- 10.56	0.95	Yes
Upslope	- 1.40	0.88	No
Recovery	2.57	0.49	Yes
Altitude	- 3.68	1.32	Yes
Cardiac index	- 2.31	0.81	Yes
Downslope * Altitude	0.90	0.09	Yes
Upslope * Altitude	2.37	0.19	Yes
Recovery * Altitude	0.67	0.08	Yes

From the table 5.3, let us first look at the value of TOI during baseline. By keeping every other co-variate the same and changing just the altitude (zero for London and 1 for Namche), the model shows that baseline TOI falls by 3.68 ($95\%CI : -6.2; -1.1$). In other words, there is a decrease in baseline TOI with ascent to altitude (Namche - 3525 meters).

The TOI during downslope was analysed next. The downslope has a coefficient of -10.56 ($95\%CI : -12.42; -8.7$). Hence, as expected, TOI during downslope is

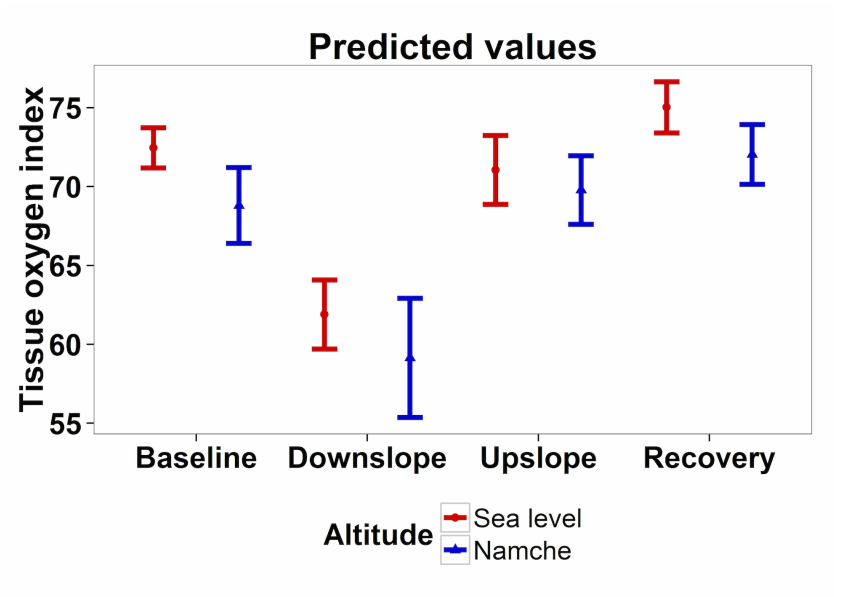
lower at London (using value of 0 for altitude co-variate) by 10.56 points compared to baseline. This is the overall downslope value.

To look at the difference in downslope at Namche compared to London, we need to look at the altitude value and its interaction with downslope. The sum of these two coefficients gives us a value of -2.8. This means TOI during downslope (marker of oxygen consumption) relative to baseline decreased on average by 2.8 units more at Namche Bazaar compared to London. The interpretation of this decrease in terms of absolute oxygen consumption value is difficult due the significant interaction between the TOI and time of recording.

To enable easy visual interpretation of results, predicted values were created from the multilevel model and plotted (Figure 5.15). These show a wide confidence interval within the 4 sections.

Figure 5.15: Predicted Tissue Oxygen Index (TOI) Derived from Multilevel Model

The TOI values were calculated as 4 parts of the NIRS VOT curve. The values in red are those for sea level and blue represents values at Namche Bazaar. The shaded circle and triangle denote mean values while the error bars represent 95% confidence intervals. There was statistically significant difference between the tissue oxygen index during downslope at sea level and Namche Bazaar.



The above analysis utilises summary values for the 4 timecodes. In order to use all the data points from throughout the NIRS VOT recording of 13 minutes the method of functional data analysis (FDA) was utilised.

5.7 Functional Data Analysis

Functional data are repeated measurements recorded as a function of a variable (such as time). Functional data analysis uses non-parametric method to build functional curves from the dataset using different basis functions. Notably, the

whole dataset (each single measurement) is utilised for the analysis. [287].
For trajectory data like NIRS VOT, one option is to employ basis splines to create functional curves. Basis splines are polynomial curve segments that are joined end to end. Together they are able to mirror the eventual curve closely. The points where the segments join are smooth. These are routinely used in all spline-based analyses.

Functional data analysis (FDA) is a complex analytical tool for large datasets using basis splines. The reasons to use the functional data analysis (despite being complex) were two fold:

1. To use all the data points in the NIRS VOT curve (about 1400 data points per recording) instead of a summary measure that will end up with 4 values for the four parts of the curve.
2. Functional data analysis (FDA) was suggested by several statisticians as the best available technique to analyse my data.

Although a simpler method might give the same result, FDA makes the results robust. In addition, as part of my learning process during this PhD, my supervisors specifically encouraged me to explore novel ways of analysing these data as part

of an intended broad training in research methodology.

5.7.1 Method

The YES2 dataset consists of 2 recordings per child across the two altitudes (London and Namche Bazaar). A total of 24 NIRS VOT curves were recorded from 12 healthy child volunteers.

The recording consists of data points at 0.5-second intervals. It is separated into four sections: baseline, drop, upslope and recovery. Data starts at 0.5 seconds and continues up to 675.5 seconds. This amounts to 1391 rows of data in each recording for each child.

Baseline data was recorded for about 2.5 minutes. Blood pressure cuff was inflated at 290.5 seconds. This was the transition from baseline to drop. The next change in the recording was at cuff deflation at 458 seconds. The following section of the recording included a rise in the tissue oxygen index (TOI) followed by normalisation.

The TOI data was converted into functional form by a two-step process. First was to create basis splines. For this, I followed regular splines methodology. [288] The FDA package in R has specific code for this process. The next step is to choose an

appropriate linear combination of these splines to result in functional TOI value [TOI (secs)]. The FDA package in R software was employed to automatically choose the splines. The final result was a TOI value that could be calculated for each time point for each recording using an equation. This process was repeated for all the 12 patient curves at the two altitudes (24 final functional curves).

5.7.2 Results

Figures 5.16 and 5.17 display the functional NIRS VOT curves of all the children in London and Namche Bazaar. These curves include the sections baseline, drop, upslope and recovery as described in figure 5.13.

Figure 5.16: Functional Data Analysis Curves of NIRS VOT in Children in London

The recording starts with a baseline section. This is followed by the downslope on inflation of the blood pressure cuff at 290.5 seconds. The cuff is deflated at 458 secs causing the upslope. The final section is the recovery phase.

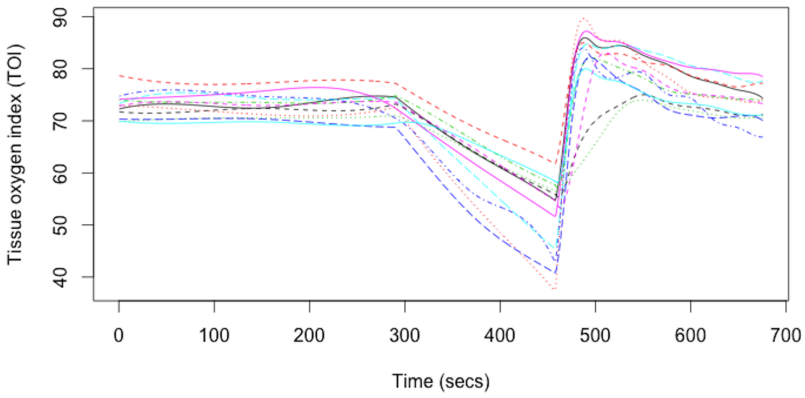
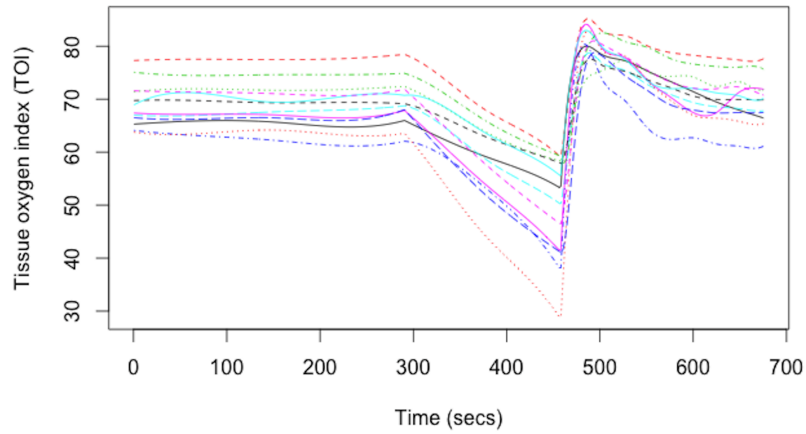


Figure 5.17: Functional Data Analysis Curves of NIRS VOT in Children in Namche Bazaar

The recording starts with a baseline section. This is followed by the downslope on inflation of the blood pressure cuff at 290.5 seconds. The cuff is deflated at 458 secs causing the upslope. The final section is the recovery phase.



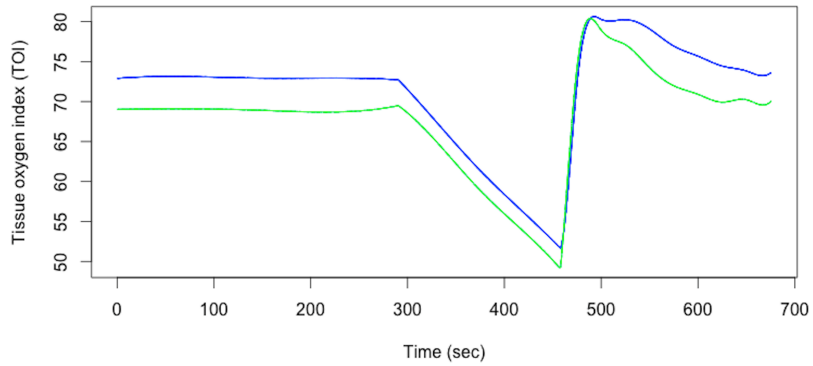
Comparison of NIRS VOT Recording Between Altitudes

The 12 individual subject data each produced a functional curve for each altitude level (London and Namche). These were combined (average of each time point) to plot a predicted average functional curve. This resulted in two curves (one for London and one for Namche) (Figure 5.19). Next, by comparing the two predicted curves I could compare the effect of altitude on TOI over the whole curve. Appendix 4 includes the R code and details of the statistical analysis.

While this technique has the advantage of comparing the whole curves of the group at each altitude, there is a loss of information in the averaging procedure and not matching of pairs that prompted a further approach.

Figure 5.18: Predicted Curve for London and Namche Bazaar

Blue curve - London, Green curve - Namche Bazaar, The predicted curves were created by using all the functional curves for recordings in London and Namche Bazaar



Functional ANOVA

The next analysis was to compare all 12 individuals functional curves produced for each of Namche and London (Figure 5.16 and Figure 5.17). These were assessed as matched pairs. The technique of functional ANOVA was employed for this analysis (point-by-point comparison of paired data).

The fundamental property of a functional curve is that each data point is represented as a function (function of time in my case). Therefore, when the two matched curves (from London and Namche for each participant) are compared we get a coefficient for each data point of the curve. Eventually I derived a mean regression coefficient curve. The confidence intervals for this regression curve were also calculated. (Figure 5.19)

Specific time values were then inputted into the final ANOVA model to derive actual TOI values for various sections of the NIRS VOT curve (baseline, lowest and drop TOI).

The forarm TOI was lower in Namche Bazaar compared to London. The baseline TOI was significantly lower in Namche Bazaar than London (-4.05, 95%CI: -3.23, -4.86, p <0.001). The lowest TOI was also significantly lower in Namche Bazaar

(-1.95, 95%CI: -1.13, -2.76, p <0.001). The DropTOI at Namche Bazaar compared to sea level was 2.1 points lower (p <0.0001). This suggests lower oxygen consumption from forearm muscle at high altitude.

Figure 5.19: Functional ANOVA

Blue curve - mean regression curve, Red curve - upper confidence interval, Green curve - lower confidence interval, Reg. Coeff. - Mean coefficient generated by comparing 12 matched pairs of functional curves between London and Namche.

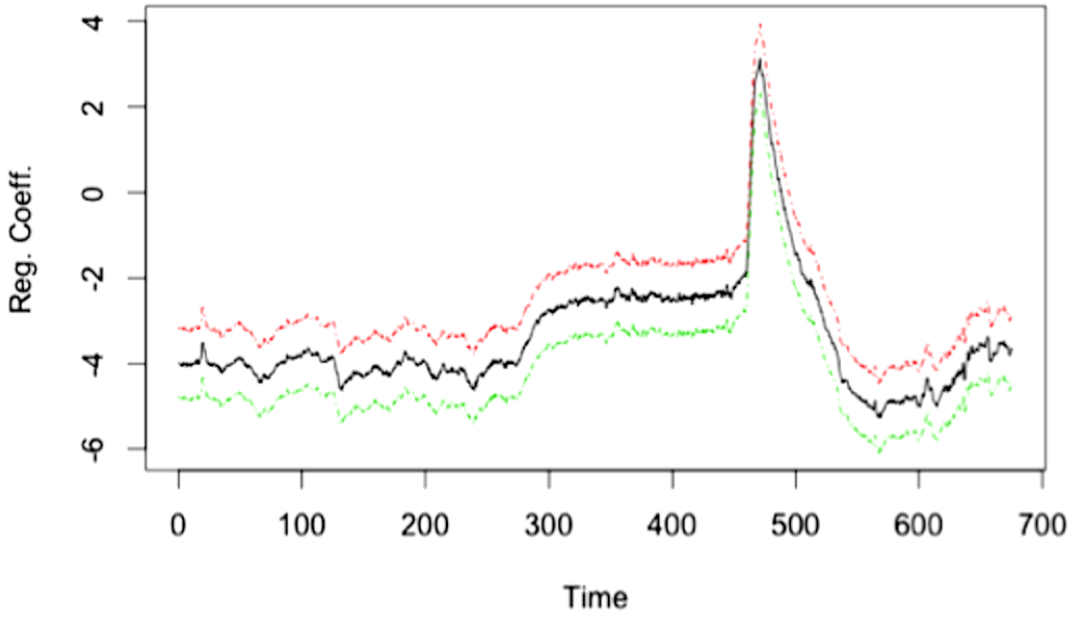


Figure 5.20: Healthy Volunteers and Investigators - Young Everest Study 2

The project was possible due to the support from the children and families who volunteered for the study. (Figure 5.20)

5.8 Conclusions

This study confirms some of the findings from previous reports. To summarise the findings reported in this chapter:

Peripheral haemoglobin oxygen saturations (at rest and after exercise) drop with an ascent to altitude. Heart rate z-scores (at rest and after exercise) and cardiac

index (at rest) do not show a statistically significant change. Respiratory rate (at rest and after exercise) increases with an ascent to high altitude. These findings support my first hypothesis that the physiological responses in children are similar to adults when exposed to hypobaric hypoxic conditions.

Baseline forearm muscle tissue oxygen index (TOI) decreases with ascent to altitude. As the partial pressure of inspired oxygen drops with altitude, it is not surprising that this decrease in baseline TOI was noted. It also correlates with the drop in peripheral oxygen saturation. The drop in peripheral oxygen saturation was clearly demonstrated in this study. The aim of the expedition was to expose healthy children to hypobaric hypoxic conditions. Therefore, the right environmental setting was achieved with this study.

Under normal conditions, oxygen delivery and consumption are uncoupled. [289] When delivery becomes limited, the relationship may become linear. The full calculation of the oxygen delivery is not possible with the parameters measured. Let us assume that the duration at altitude was not long enough to cause a raise in haematocrit. Consequently, the product of cardiac index and peripheral oxygen saturation could be used as a surrogate marker for oxygen delivery (let us call it 'cardiacox'). As peripheral oxygen saturation drops with altitude and cardiac

index remains the same, *cardiaxox* drops. Consequently, oxygen delivery does decrease under hypobaric hypoxic conditions, assuming haematocrit does not change. Is this reduction enough to impact oxygen consumption?

To answer this question, I measured Drop TOI (forearm oxygen consumption). Drop TOI decreases by 2.1 points at altitude compared to sea level in healthy children. This might suggest a more efficient mitochondrial function. Interestingly, adult lowlanders examined under the Xtreme Everest study did not show a reduction in oxygen consumption at altitude. [265] This variation in results might be due to a few reasons. First and foremost, I may be over interpreting the results from YES2 (with a small sample size and short duration at moderate high altitude). Second, adults have relatively more subcutaneous fat and this might impede accurate TOI measurement. Third, the adult study had a slightly different placement of the NIRS probe (thenar compared to my lateral aspect of forearm position). Fourth, they noted an increased rate of recovery suggesting a change in vasomotor response at altitude. This could be due to age-related vascular changes in adults. In addition, children have a higher sympathetic activity that might mean they have an inherent increased vasomotor tone. A study in Sherpas has suggested an more efficient mitochondrial function. [186] This might help explain my YES2 Drop TOI results.

Cerebral NIRS does not change with altitude on either side of the cerebral circulation. In addition, nearly all children were able to complete the trek (one was repatriated due to Salmonella gastroenteritis). These findings suggest that it is safe for children of ages 8-16 years to trek to an altitude of 3525 metres. The YES study noted a variation in both middle cerebral artery and anterior cerebral artery blood flow with cerebral transcranial doppler measurements. [290] Another member of the YES2 team has recorded these parameters. I intend to analyse these with the cerebral NIRS values to look for correlations.

Heart rate z-score did not change with altitude. This result is different to the previous reports from the YES study. [283] The obvious limitations of small numbers, short duration at altitude and moderate high altitude does not explain the difference in results as both studies have the same limitations. The only difference that could be significant is that Scrase et al presented absolute numbers whilst I present z-scores. z-scores are a statistically validated method of presenting physiological data.

The rise in respiratory rate might be in tandem with a need for increase in minute ventilation for improving gas exchange at the lungs. I do not have blood gas values

to investigate PaO_2 and PCO_2 values. These might have given added insight into gas exchange and function of the respiratory system.

None of the children in my study showed any signs of high altitude pulmonary edema or high altitude cerebral edema. This might be due to the gradual ascent profile and the moderate high altitude reached. It would have been interesting to investigate if any of these children carried the I-allele of ACE gene. Unfortunately, I was unable to test this given the invasive nature of this test.

Limitations

The main aim of the study was to explore hypoxic adaptive mechanisms. However, with a short duration of ascent and stay at altitude (5 days) it is unclear if the long-term adaptive responses would have started to occur. A trek to a higher altitude and a stay for several weeks would be ideal to investigate these mechanisms. Unfortunately, safety risks restricted our expedition to become more pragmatic.

The aim of exposure to altitude was to reduce oxygen delivery. Although I did not measure all the values needed for calculation of oxygen delivery, my surrogate marker suggests a drop with ascent to altitude. Whether this drop was significant enough to affect the oxygen delivery-consumption relationship is unclear. The

oxygen consumption does seem to reduce at altitude. However, there are several limitations such as small number, short duration and moderate altitude that limit the robustness of results. Therefore, to be certain of a change in the oxygen delivery - consumption relationship further research would be necessary.

Lower age groups (age 1-5 years) were not tested in this study. The aim was to utilise the results from this study to understand the patho-physiology of the paediatric intensive care patient. The majority of patients (about 80%) in UK PICUs are less than 5 years of age. This considerably limits the potential extrapolation of YES2 findings to the PICU population.

Another limitation of this study was the need to restrict testing to non-invasive techniques. This led to utilising NIRS VOT that at best is only able to give a snapshot view into regional (forearm muscle) oxygen consumption. With NIRS VOT recording I was unable to measure dynamic changes in cardiac output. This would have enabled a better understanding of the oxygen consumption-delivery relationship.

The confidence intervals with cardiac index measurements shortened in the later part of the expedition. This may be due to improvement in my technique and also

because the children were aware of the position needed for effective test results. The small sample size makes it difficult to be certain if the cardiac index is increasing with altitude or not.

Ambient and peripheral skin temperature can also have a major effect on the NIRS VOT measurements. [291] The room temperature setting of 20 °C, for the Young Everest Study 2, standardised the effect of ambient room temperature.

Chapter 6

Variation in Oxygen Consumption During Paediatric Critical Illness

I have previously discussed the ability of humans to adapt to hypoxic conditions and some of the mechanisms that may be involved in these adaptations. The possibility that similar processes may be involved during evolving critical illnesses in which oxygen delivery maybe abnormal was a key motivation behind the work presented in this thesis.

In chapter 1, I described the methods that might be used to measure oxygen consumption (section 1.5). The tissues from which oxygen consumption is routinely measured were also detailed. Here, I describe the current understanding of oxygen consumption - oxygen delivery relationship.

6.1 Oxygen Consumption - Oxygen Delivery Relationship

Critical illness challenges physiological mechanisms that balance oxygen delivery and consumption. Initial studies in critically ill adults with acute respiratory distress syndrome reported a linear relationship between oxygen consumption (VO_2) and oxygen delivery (DO_2). [292] [293] In other words, oxygen consumption was limited by the supply of oxygen to tissues. Many potential mechanisms might contribute to this relationship including macro and microvascular changes (altered regional blood flow and an increased capillary density). [294] However, in adult patients without respiratory failure undergoing surgery the two variables were noted to be independent above a critical threshold DO_2 value. [295]

After the linear relationship between VO_2 and DO_2 was noted, efforts were directed at optimising oxygen delivery in order to increase oxygen consumption. These studies displayed mixed results. [296] Soon researchers realised that the linear association was not true in all clinical scenarios. Only a proportion of patients with lower than normal oxygen delivery were benefited by therapeutic strategies that augment this parameter. Hanique et al demonstrated that the delivery-consumption dependence was more likely to be a consequence of mathematical coupling. This association was only noticed when VO_2 and DO_2 were calculated rather than being directly measured. [297] As discussed in chapter 1

(section 1.6.1), oxygen delivery is calculated from the equation:

DO_2 = CO x CaO_2 measured in mls/min

where

CaO_2 (arterial oxygen content) = (SaO_2 x Hb x 1.39 + 0.0031 x PaO_2) measured in ml/dl,

SaO_2 denotes haemoglobin oxygen saturation,

Hb denotes haemoglobin concentration,

PaO_2 denotes partial pressure of arterial oxygen and oxygen consumption is calculated by the equation:

VO_2 = CO x AVD measured in mls/min

where

AVD (Arterial-Venous oxygen difference) = CaO_2 - CvO_2 measured in mls/dl,

CvO_2 = SvO_2 x Hb x 1.39 + 0.0031 x PvO_2 measured in mls/dl

The above equations show that oxygen delivery and oxygen consumption are derived from shared variables. Therefore, the calculated variables co-vary. Crucially when measured, rather than derived this relationship changes. [289] Supply dependent oxygen consumption has not been demonstrated in children and

is now thought to be an exception rather than a typical state in adult critical illness. [298] [299]

Tonsoni recently showed that VO_2 and DO_2 are independent in children during extracorporeal membrane oxygenation (ECMO). [289] They manipulated the DO_2 directly by alternatively decreasing and then increasing the flow rates by 25% from the baseline on ECMO. The VO_2 was measured using respiratory mass spectrometry. From 11 patients, they demonstrated no change in VO_2 between the baseline values and those recorded after decreasing or increasing DO_2 (P = 0.18). The caveat to note is that these children were already fully resuscitated.

Therefore the current view is that while in a steady state, VO_2 and DO_2 are independent at the whole body level. However, *in-vitro* studies with hepatocytes have reported a decrease in oxygen consumption with a reduction in oxygen supply. [300] De Backer observed a similar linear correlation between VO_2 and DO_2 in the hepato-splanchnic circulation in a subgroup of 42 septic patients. This suggests that regional oxygen delivery dependent oxygen consumption might exist in some clinical scenarios and perhaps in some organs. [301]

The recognition that VO_2 and DO_2 are uncoupled at the whole body level has led to a shift in management aims. The focus has turned to the measurement of oxy-

gen consumption rather than manipulating delivery. As was mentioned earlier in section 1.5, the oxygen consumption may be measured by invasive or non-invasive techniques. The serum lactate, mixed venous saturation and veno-arterial PCO_2 difference from blood may be employed as surrogate markers of whole body oxygen consumption. [302] [303] Further, regional oxygen consumption can be measured from blood cell components and tissues such as muscle and skin using a technique that has evolved from the initial Clarke oxygen electrode. [76] [78] In chapter 3, it was shown that near infrared spectroscopy with a vascular occlusion test may have some utility as a surrogate marker of regional (forearm) oxygen consumption. Although there have been advances in techniques that measure oxygen consumption, clinicians still primarily employ peripheral oxygen saturation as a target to deliver resuscitation and further management in the critically ill patient. In mechanically ventilated patients a lower oxygen saturation threshold is usually set at 92%. However, an upper threshold is not routinely prescribed, and observational studies suggest that upper thresholds are not adhered to even when set. [304] Clinicians seem to reduce the upper limit of oxygen saturation as the PaO_2/FiO_2 ratio decreases in acute lung injury. This response might be attributed to the perceived harm due to hyperoxia or an awareness of higher ventilator settings that might contributor to lung injury. [17] A similar response has been noted in children with acute respiratory distress syndrome. [305] My systematic review (chapter 3) does

not clearly point towards harm due to hyperoxia. Hence, the correct oxygen saturation range is still unclear. The impact of varying oxygen saturation, although not noted as a mortality signal, might be apparent in the morbidity profile. Morbidity has been suggested as a more relevant outcome measure compared to mortality in the paediatric intensive care unit. [306] Recent consensus guidelines concur with this recommendation. [307] The morbidity following critical illness is dependent on the severity of organ failure. Furthermore, acute mitochondrial dysfunction may contribute to multi-organ failure during critical illness. Let us now discuss the evidence behind mitochondrial function in critical illness.

Although it is far from certain what exactly is the accepted signature of hypoxic adaptation, I will discuss the most up to date understanding of this mechanism next.

6.1.1 Lessons from Genetics

Numerous genetic studies have compared Tibetan highlanders and low landers from across the world. Three genes - EPAS1, EGLN1 and PPARA are highly expressed in Tibetans. [308] These modulate and are in turn controlled by the hypoxia inducible factor system (HIF). As noted in chapter 1 (section 1.5.1), HIFs are the key transcription factors regulating oxygen sensing and homeostasis in

humans. Through the HIF pathway, these genes alter erythropoiesis, the oxygen cascade and other metabolic processes. These genes may represent evolutionary modifications in hypoxia-adapted humans. [309]

6.1.2 The HIF System and Mitochondrial Function

Activation of the HIF system is required for mitochondrial homeostasis. [310] Reciprocally, mitochondrial oxygen consumption is necessary for the induction of HIF system. [311] The HIF system modulates mitochondrial function under hypoxic condition by three main modes:

1. Stimulating energy production from glycolysis,
2. Suppressing the Krebs cycle and oxidative phosphorylation and
3. Reducing mitochondrial ROS production. [312]

Furthermore, HIF limits mitochondrial biogenesis. Consequently, mitochondrial mass and oxygen consumption is reduced. These interactions result in a more efficient energy production system that also protects the cell from hypoxic damage. [313] How might this knowledge help manage the critically ill patient?

6.1.3 Mitochondrial Function in Critical Illness

Critically ill adults seem to have an impaired utilisation of oxygen with decreased production of ATP. Brealey et al observed reduced ATP concentration in skeletal muscle of septic critically ill patients who died compared to controls (p=0.05) and those who survived (p=0.0003). The reduced ATP concentration might suggest mitochondrial dysfunction. [314] Recovery from this mitochondrial dysfunction may be important for outcome.

Carre et al noted a positive correlation between survival and early activation of mitochondrial biogenesis. [315] Activation of biogenesis suggests protein synthesis and up-regulation of gene expression.

In a landmark paper, time-series gene expression profile was performed following administration of a dose of endotoxin in 8 healthy volunteers. A transient bioenergetics failure (decreased expression of oxidative phosphorylation genes - OXPHOS) was demonstrated by using a genome-wide network analysis. [316] The transient nature of the bioenergy failure is of particular interest to the intensivist. An understanding of why this energy failure might be transient and the pathways involved in its recovery could focus therapeutic interventions in the critically ill patient.

Weiss et al have investigated the theory of down regulation of mitochondrial gene expression in the paediatric septic shock population. By employing gene expression analysis on data from 180 patients, they were able to demonstrate differential expression in 118 nuclear coded mitochondrial genes compared to controls. Of interest to my project, the OXPHOS gene set was the most down regulated among the panel. [317]

To summarize, critically ill patients have mitochondrial dysfunction probably secondary to down regulation of OXPHOS gene expression. Their survival seems to be associated with the recovery of mitochondrial function. This recovery might be modulated (positively adapted) by the hypoxia-sensitive HIF system. Let us now look at the potential response to hypoxia in the critically ill patient.

6.1.4 How Might Hypoxic Adaptation Present in the Critically Ill Patient?

If the hypothesis of hypoxic adaptation were to be true, one might observe the OXPHOS function to drop initially, stabilize, start to increase and plateau with progression of critical illness. The eventual state might be of an augmented OXPHOS function in the context of relative lower oxygen-requirement state.

Sjvall et al investigated the functional correlate of the change in OXPHOS gene expression. They explored platelet mitochondrial function in septic patients and healthy controls. The Oxidative phosphorylation system was interrogated at 3 time points day 1/2, day 3/4 and day 6/7 with high-resolution respirometry. They observed increased uncoupling by day 3/4. Interestingly, non-survivors demonstrated significantly more uncoupling compared to survivors. [172] These findings may concur with the hypoxic adaptation theory discussed above. The uncoupled state is transient causing OXPHOS dysfunction. In patients who survive, this state transitions into a steady stay and finally increased coupling. The result is an improvement in OXPHOS function that is mandatory for survival. The Weiss group has reported similar results in paediatric septic shock. [174]

This chapter describes my attempt to investigate changes in oxygen consumption in critically-ill children with time.

Three techniques are included:

(1) Serial near infrared spectroscopy with a vascular occlusion test (NIRS VOT) to assess *in-vivo* muscle oxygen consumption in critically ill children admitted to paediatric intensive care (Study 1: The serial NIRS VOT study).

(2) Serial measurement of molecular oxygen consumption of peripheral blood

mononuclear cells using a high resolution respirometer (Oroboros-2K analyser - Study 2: The O2K study).

(3) Serial analysis of oxidative phosphorylation gene expression profiles of children admitted with meningococcal sepsis using Gene Set Enrichment Analysis (GSEA) (Study 3: The OXPHOS gene expression study).

6.2 Methods

Hypotheses:

1. Serial measurements of drop Tissue Oxygen Index (TOI) during the vascular occlusion test will not alter during the time course of critical illness in children.

2. Serial measurements of peripheral blood mononuclear cell mitochondrial oxygen consumption will not alter during the time course of critical illness in children.

3. Gene expression of oxidative phosphorylation genes will not change in the first 48 hours of paediatric critical illness.

6.2.1 Study Design:

Study 1: The serial NIRS VOT study

The serial NIRS VOT was a prospective observational study.

Study 2: The O2K study

The O2K study was a prospective observational study.

Study 3: The OXPHOS gene expression study

The OXPHOS gene expression study was an *in-silico* analysis of previously collected gene expression data by Dr Kwan and others.

6.2.2 Inclusion Criteria

Study 1: The serial NIRS VOT study

All children with critical illness admitted to the paediatric intensive care unit with a diagnosis of systemic inflammatory response syndrome (SIRS) and expected to be ventilated for >3 days were eligible.

SIRS was defined as a patient who has two or more of the following parameters:

1. Abnormal white cell count (<4 or >12x10^9 per l)

2. Temperature instability (<36 or >38.5 °C)

3. Tachycardia (mean heart rate >2 standard deviations above normal for age) or bradycardia (mean heart rate <10th percentile for age) in children <1 yr old.

4. Mean respiratory rate >2 standard deviations above normal for age or mechanical ventilation for an acute process. [318]

The ideal population to study would be those with problems with oxygenation. There were four main reasons to choose children with SIRS for this preliminary study. They were:

1. Systemic inflammatory response syndrome is the underlying process for most of the causes of admission to intensive care. In addition, previous studies have shown that the SIRS population can be considered a homogenous group for analysis. [319] The children with oxygenation problems could be expected to have an exaggerated response compared to those in the SIRS population.

2. The SIRS criteria could be used as a marker of severity of illness. Therefore, these children would be expected to need comparatively longer duration of mechanical ventilatory / inotropic support. [318] This will enable longer data capture for this study.

3. Nearly 60% of children admitted to the paediatric intensive care unit fit the criteria for SIRS. [320] This lent a large patient cohort for recruitment.

4. SIRS patients may have more tissue hypoxia rather than alveolar hypoxia. This would be ideal to the investigation of tissue oxygen consumption.

Study 2: The O2K study

All children admitted to the paediatric intensive care unit with a diagnosis of the

systemic inflammatory response syndrome (defined as for study 1 above) with one or more organ failure (defined as Paediatric logistic organ dysfunction 2 score ≥7) were eligible for recruitment.

Study 3: The OXPHOS gene expression study

All children with a presumptive diagnosis of meningococcal sepsis admitted to the paediatric intensive care unit in Nottingham university hospital were eligible. Blood samples were collected, from 5 children with Meningococcal sepsis, at 6 time points: 0, 4, 8, 12, 24, and 48 hours from dose of antibiotics. Details of the sample preparation and analysis are published in the article by Kwan and include in the appendix to this thesis. [321]

6.2.3 Exclusion Criteria

Study 1: The serial NIRS VOT study

1. Children who were not mechanically ventilated
2. Ex-premature infants (<30 wks gestation at birth)
3. Children with suspected mitochondrial/metabolic disease

Study 2: The O2K study

1. Children with no invasive line for access
2. Children transferred from other intensive care units after a prolonged admission
3. Children with traumatic brain injury
4. Children with ethical issues in their management, such as withdrawal of intensive care
5. Children with suspected mitochondrial disease

Paediatric Logistic Organ Dysfunction 2 score

The Paediatric Organ Dysfunction score (PELOD) is a descriptor of the severity of multi-organ dysfunction. The PELOD score was not created to be a predictor of mortality. The scoring system includes variables selected by a Delphi consensus method. The alternative strategy of estimating organ dysfunction - PEdiatric Multiple Organ Dysfunction (PEMOD) system included the probability of mortality. However, the PEMOD system was unable to discriminate between patients as well as the PELOD score. In addition, the use of a logistic regression analysis enables the PELOD to differentiate between the individual organ dysfunction and the varying severity levels within each organ dysfunction system. [322] The score was validated in 1806 patients' data collected in a prospective observational cohort study conducted across 7 paediatric intensive care units. [323] The updated

version of the PELOD score is the PELOD2. [324] It incorporates variables accounting for each of the organ systems assessed. These are Glasgow Coma Scale (GCS), pupillary reaction, Lactate level, mean arterial pressure, creatinine level, PaO_2/FiO_2 ratio, $PaCO_2$, ventilation status, white cell count and platelet count. A daily estimation of PELOD score has been observed to be different between survivors and non-survivors. [195] Therefore, the recently updated PELOD2 score was used to objectively quantify the severity of illness in the recruited children in both the serial NIRS VOT and O2K studies.

The GCS included in the scoring of PELOD2 is a composite measure. The variables that make up the GCS include eye opening, a motor and a verbal component. Sedated and mechanically ventilated patients are non-verbal, may not be able to open their eyes and may not be able to move (if heavily sedated or paralysed). Hence, it is not possible to calculate the GCS in this patient population.

Table 6.1: Paediatric Logistic Organ Dysfunction 2 score

Organ dysfunction	**Severity levels 0**	**1**	**2**	**3**	**4**	**5**	**6**
GCS	>11	5-10			3-4		
Pupillary reaction	Both reactive					Both fixed	
Lactate (mmol/l)	<5	5-11				>11	
Mean arterial pressure (mmHg)							
0-1 month	≥46		31-45	17-30			≤16
1-11 months	≥55		39-54	25-38			≤24
12-23 months	≥60		44-59	31-43			≤30
24-59 months	≥62		46-61	32-44			≤31
60-143 months	≥65		49-64	36-48			≤35
≥144 months	≥67		52-66	38-51			≤37
Creatinine							
0-1 month	≤69		≥70				
1-11 months	≤22		≥23				
12-23 months	≤34		≥35				
24-59 months	≤50		≥51				
60-143 months	≤58		≥59				
≥144 months	≤92		≥93				
PaO_2/FiO_2 ratio	≥61		≤60				
$PaCO_2$ mmHg	≤58	59-94		≥95			
Invasive ventilation	No			Yes			
White cell count	>2		≤2				
Platelet count	≥142	77-141	≤76				

Legend: Reproduced from the article by Leteurtre. [324], GCS - Glasgow Coma Scale, PaO_2 - arterial partial pressure of oxygen, FiO_2 - fraction of inspired oxygen, $PaCO_2$ - arterial partial pressure of carbon dioxide, creatine measured in micromol/l, white cell count and platelets measured in x $10^9/l$

The diagnosis and physiological parameters such as heart rate, blood pressure and oxygen saturation were collected. For the serial NIRS VOT and O2K studies, the durations of illness for the patients varied. I was unable to account for these variations just from the history. Therefore, a pragmatic time code was employed. 'Day 0' was defined as the period from admission to the paediatric intensive care unit to midnight of that day.

Research and Ethics approval

These three studies each obtained formal research ethical and local research governance approvals. The serial NIRS VOT and O2K studies were approved in a combined application (NRES-Hampstead -14/LO/1819). The approvals for the gene expression study were obtained by Dr Rashid from Queens Medical Centre PICU, Nottingham University committee (REC reference - 05/Q2403/53). Signed informed consent was obtained from a parent or guardian for each child included in these studies.

The ethics application for the serial NIRS VOT and O2K studies included a statistical analysis plan acknowledging the limited information on which power calculation and sample size estimates could be made with these novel studies.

Tests performed in the studies

Study 1: The serial NIRS VOT study

Near infrared spectroscopy with a vascular occlusion test (NIRS VOT) was performed daily to measure the forearm muscle oxygen consumption (recorded as the drop in tissue oxygen index Drop TOI, detailed description in chapter 2.1). The cardiac index was measured using the suprasternal ultrasound cardiac output monitor (USCOM - chapter 2.2).

Study 2: The O2K study

Near infrared spectroscopy with a vascular occlusion test (NIRS VOT) was performed daily to measure the forearm muscle oxygen consumption (recorded as the drop in tissue oxygen index Drop TOI, detailed description in chapter 2.1). Daily analysis of peripheral blood mononuclear cells oxygen consumption using the Oroboros analyser was performed. The cardiac index was measured using the suprasternal ultrasound cardiac output monitor (USCOM - chapter 2.2).

I ascertained that the recruits were clinically stable (after discussion with treating medical and nursing team) during the NIRS VOT measurements (both study 1

and 2) and blood sampling (O2K study). This ensured that there were no changes in infusions of vasoactive agents, no fluid boluses administered and no changes in ventilatory parameters whilst the tests were performed.

Oxygen delivery

The oxygen delivery was estimated by the equation:

$$DO_2I = CI \times CaO_2 \tag{6.1}$$

where

CI denotes cardiac index (measured using USCOM) and

CaO_2 denotes oxygen content of arterial blood measured in mls of $O_2/decilitre$.

The arterial oxygen content was calculated by the equation:

$$CaO_2 = (1.36 \times Hb \times SaO_2) + (0.0031 \times PaO_2) \tag{6.2}$$

where

Hb denotes haemoglobin measured in grams/decilitre,

SaO_2 denotes saturations from arterial blood gas measured as percentage,

PaO_2 denotes arterial partial pressure of oxygen measured in mmHg and

Haemoglobin concentration, SaO_2 and PaO_2 were recorded prospectively with each recording of the NIRS VOT to enable the oxygen delivery calculation.

Power calculation

Study 1: The serial NIRS VOT study

A comparison of two means with two samples and two-sided equality formula was employed for the sample size calculation. The power was set at 80% and alpha error was set at 5%. The mean and standard deviation for sample A was chosen from NIRS VOT measurements in healthy children at sea level (mean: 21.1, SD: 7.09, from the YES2 study). The mean for sample B was empirically chosen as a reduction of 25% from healthy controls. This was thought to be a clinically relevant change in oxygen consumption. Around 22 patients were needed for this study. This is necessarily an approximation because of the novel nature of this work meaning that prior estimates of likely changes in NIRS VOT could not be known in advance nor could the variance of these values. A further limitation is that this estimation ignores the complexity of a time series dataset.

Study 2: The O2K study

A formal power calculation was difficult as the variability of oxygen consumption measurement from the O2K analysis in critically ill children was unknown. Weiss et al have analysed oxygen consumption in peripheral blood mononuclear cells in critically ill children on day 1-2 and day 6-7 after admission. [174] If I were to achieve the same percentage change of oxygen consumption as in their report with a power of 80% and alpha set at 5%; I will need 3 patients for a pairwise comparison of day 1 to day 5. As it was unclear if peripheral blood mononuclear cell extraction would be successful in all patients, I set out to recruit 10 patients.

Study 3: The OXPHOS gene expression study

This study was an *in-silico* analysis of the dataset from Dr Kwan's study. [321] The data from 5 patients' gene expression was available for analysis.

6.3 Statistical Analysis

The admission baseline characteristics and clinical information are presented as summary statistics (median with interquartile range or absolute number with percentages) for both the serial NIRS VOT study and O2K study.

6.3.1 Study 1: The Serial NIRS VOT Study

These data were analysed as follows:

1. Univariate distribution of the summary variable DropTOI was compared with age, sex, weight, day of illness, diagnosis, PELOD2 score, baseline Tissue Oxygen Index (TOI), cardiac index, CaO_2, haemoglobin, PaO_2, SaO_2, DO_2I and status at discharge.

2. A multilevel linear regression model was employed to analyse DropTOI. The DropTOI, as the outcome measure, was nested within individual patients. The co-variates with a statistically significant univariate relationship were included in the model. The results are presented as individual non-standardised coefficients with confidence intervals.

3. Multilevel functional data analysis (FDA) was employed by using the whole curve of NIRS VOT. The NIRS VOT curve has data points recorded at frequency of 2 per second. However, this high resolution imparts considerable roughness to the curve. In addition, the curve has 4 phases with different slopes. The FDA technique is able to account for all the above unique issues. First, it utillises all the individual data points. Therefore, there is no loss of data. This would have been inevitable if I had used a summary measure. Second, the roughness of the individual curves is counteracted by incorporating a smoothing constant. Finally, the varying slopes are accounted for by using a spline based approach.

The FDA has found major applications in studies involving brain imaging such as the study of white matter structures in multiple sclerosis and the geometry of cerebral aneurysms. [325] Further details of the code used are included as an appendix.

Functional data analysis of the NIRS VOT curves

Functional data analysis as detailed in section 5.6 was employed. To model the NIRS VOT functional curves, a cubic spline of the 4th order was employed. A total of 20 basis splines were created with interior breaks (16) at specific points. The NIRS VOT recordings consisted of data points at 0.5-second intervals. It is separated into four sections: baseline, drop, upslope and recovery. Data started at 0.5 seconds and continued up to 697.5 seconds. This amounted to 1395 rows of data in each recording for each child. The baseline data was recorded for about 2.5 minutes. The blood pressure cuff was inflated at 302.5 seconds. This was the transition from baseline to drop. The next change in the recording was at cuff deflation at 461.5 seconds. The following section of the recording included a rise in the tissue oxygen index (TOI) followed by normalisation.

6.3.2 Study 2: The O2K Study

Admission age, weight and cardiac index are presented as summary statistics. This is as median with interquartile range if not normally distributed. PELOD2 scores

have been reported as a range.

The variables that are relevant for the power of the study are:

1. The wide variety of underlying diagnoses and associated clinical severity of recruits.

2. The comparison of NIRS VOT and O2k analysis was attempted with the Bland-Altman method for limits of agreement. But the variability of oxygen consumption measurement from O2K in this population is unknown. Further, oxygen consumption measured from different tissues makes the Bland-Altman method inappropriate.

The measurements I made from the O2K experiments are leak control ratio (LCR), net routine control ratio (NetRCR) and coupling efficiency. As detailed in chapter 2 (section 2.3), LCR is the ratio of LEAK respiration and ETS capacity, NetRCR is the oxygen consumption due to phosphorylation after normalising for leak respiration and coupling efficiency is the amount of coupling occurring when normalised for leak respiration.

The change of netRCR and LCR with duration of illness was analysed as a multilevel model. The NetRCR and LCR were outcome variables and day of illness was the fixed effect co-variate while the patient was set at level 2. Analysis of variance of normalised NetRCR, LCR and coupling efficiency to DropTOI was employed to investigate the relationship between O2K data and NIRS VOT measurements. To

normalise NetRCR, LCR and coupling efficiency, values were divided by the first recorded measurement for the patient.

6.3.3 Study 3: Gene Set Enrichment Analysis for OXPHOS Gene Expression Study

The time-course of the OXPHOS gene expression in peripheral blood was explored with Gene Set Enrichment Analysis (GSEA, Broad institute, MIT), at 0, 4, 8, 12, 24, and 48 hours from time of dose of antibiotics in the emergency department. [326] Extracted RNA was hybridised in Affymetrix microarrays by Dr Kwan. Methodological details are published in her article. [321] The dataset is available to download from the European Bioinformatics Institute database (ArrayExpress id: E-MEXP-3850). The blood sampled in the emergency department was assigned as time 0. This was a realistic reference point, but it was noted that children may have been unwell for varying periods before presentation to the emergency department.

This technique was chosen, as it is the ideal technique for analysing time series data. The GSEA ranks gene expression data according to a predetermined class file. It looks for the distribution of a curated gene set (with close functional activity) in this ranked list. The report consists of the correlation to the class file and orders gene sets according to their Normalised Enrichment Score (NES - nor-

malised for the length of gene set), nominal p-value, and False Discovery Rate (FDR).

Continuous phenotype class file

As the gene expression profile was a time series data, a continuous phenotype label with a decreasing profile was employed. The class file utilised for the analysis was in the following 3 line code:

#numeric

#LineralyDecreasingProfile

48 44 40 36 24 0 48 44 40 36 24 0 48 44 40 36 24 0 48 40 36 24 0 48 44 40 36 24 0

The linearly decreasing profile code denotes the time points at which the gene expression profile was measured. As the blood samples were collected at 0,4,8,12,24 and 48, an inverse of this was employed for the decreasing profile. The recurring numbers are for the 5 different patients. The code was written as per the rules set by GSEA. [326]

Gene set file

The publically accessible curated C2 gene sets from version 4 of the molecular signature database (MSigDb) were utilised as the gene set file. [326] A total of

3655 gene sets (includes 20693 genes) were used in this analysis. The expression profile of Reactome tricarboxylic acid cycle and respiratory electron transport chain (RETC) gene set was investigated. This dataset contains 70 genes. The full list of the genes included in this dataset is in appendix 3.

6.4 Results

6.4.1 The Serial NIRS VOT Study

Ninety-four NIRS VOT measurements were undertaken in 35 children accounting for 94 recordings. Eighty-two percent (29/35) of the children survived to intensive care unit discharge. Just over half the children recruited were admitted with sepsis (51.4%). The Figure 6.1 below notes the timing and the number of repeats for each patient.

Figure 6.1: Timing and number of repeats of NIRS VOT

Patient ID	Day 0	Day 1	Day 2	Day 3	Day 4
GICS101		x	X		X
GICS102			X	X	X
GICS103		X	X	X	
GICS104			X	X	X
GICS105		X	X		X
GICS106	X	X			
GICS107		X		X	
GICS108		X	X		X
GICS109		X			X
GICS110		X	X		
GICS111	X	X			
GICS112	X		X	X	
GICS113		X	X		X
GICS114		X	X		
GICS115			X		X
GICS116	X		X	X	X
GICS117		X	X	X	
GICS118	X	X			
GICS119	X	X			
GICS120		X			
GICS121		X	X		X
GICS122			X	X	
GICS123		X			X
GICS124	X	X	X		
GICS125	X	X	X		
GICS126	X	X	X	X	
GICS127	X		X		X
GICS128	X	X	X	X	
GICS129	X				
GICS130			X	X	X
GICS131		X	X	X	
GICS132	X				
GICS133	X		X		X
GICS134	X	X	X	X	X
GICS135	X	X	X	X	

A GCS of 3 was used for all patients in my study cohort as they were all sedated and mechanically ventilated. This gave a baseline score of 4 for all patients. For example, patient 101 (serial NIRS VOT study) was assessed on 'day 1'. Table 6.2 illustrates the calculation of the PELOD2 score from individual variables.

Table 6.2: An illustrative example of a calculation of the Paediatric Logistic Organ Dysfunction 2 score

Characteristic	**Patient's value**	**Score**
GCS	3	4
Pupillary reaction	Bilaterally equal and reactive	0
Lactate	2 (< 5)	0
Mean arterial pressure	77 mmHg (>cutoff 3)	0
Creatinine	22 (<cutoff 1)	0
PaO_2/FiO_2 ratio	325 (>60)	0
$PaCO_2$	41.8 mmHg (<58)	0
Invasive ventilation	Yes	3
White cell count	8.35 (>2)	0
Platelet count	205 (>142)	0
Total score		7

Key: PELOD2 - Paediatric Logistic Organ Dysfunction 2 score, GCS - Glasgow Coma Scale, PaO_2 - Arterial partial pressure of oxygen, FiO_2 - fraction of inspired oxygen, $PaCO_2$ - Arterial partial pressure of carbon dioxide

The PELOD2 score ranged from 7-16 (Figure 6.2). Baseline characteristics and clinical information are presented in table 6.3.

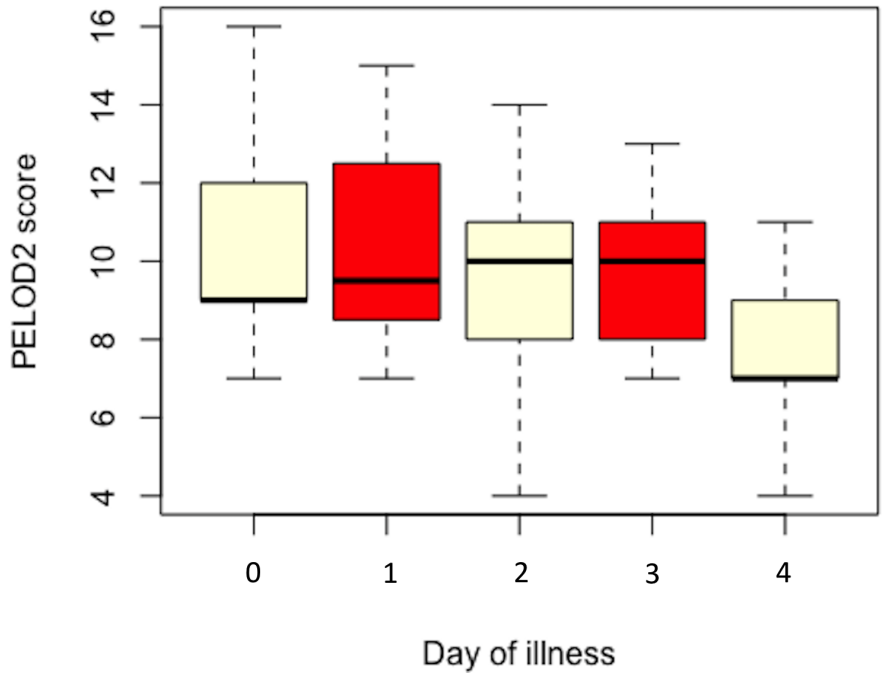
Table 6.3: Details of children recruited to serial near infrared spectroscopy with a vascular occlusion test study

Characteristic	**Value, n = 35**
Age (months)	49.2 (IQR:14.5-110)
Weight (Kgs)	15 (IQR:10-28.5)
Cardiac index $(L/min/m^2)$	5 (IQR:4-5.7)
Sex	
Male	19 (54.2%)
Female	16 (45.8%)
Diagnosis	
Post-surgical	5 (14.2%)
Respiratory	8 (22.8%)
Cardiac	1 (2.8%)
Sepsis	18 (51.4%)
Others	3 (8.5%)
PELOD2 (range)	7-16
Alive at ICU discharge	29 (82.8%)

Legend: Characteristics are presented as median with interquartile range (IQR) or absolute number with percentages. PELOD2 - Pediatric logistic organ dysfunction score 2, ICU - Intensive care unit. PELOD2 was calculated at each point of near infrared spectroscopy with a vascular occlusion test recording.

Figure 6.2: Distribution of Pediatric Logistic Organ Dysfunction 2 score (PELOD2) in the First 5 days of Illness

Box plot of the variation in the PELOD2 score. The bold horizontal line within the box plots represents median values. The box represents the interquartile range. The error bars denote the range of values.



The observed median Drop TOI was 21.1 (IQR: 15.6-23.8) on day 0 of illness. This decreased to 17.6 on day 2 (IQR: 14.1-20.0) and increased again on day 4 to 19.8 (15.2-23.3).(Table 6.4, figure 6.3 and 6.4)

Table 6.4: Median Tissue Oxygen Index values during the first 5 days of critical illness

Day of illness	**Baseline TOI**	**Drop TOI**
Day 0	70.9 (65.7-75.6)	21.1 (15.6-23.8)
Day 1	74.9 (71.9-78.6)	18.1 (15.8-24.2)
Day 2	73.0 (68.5-78.9)	17.6 (14.1-20.0)
Day 3	76.9(73.2-81.0)	18.4 (17.0-19.6)
Day 4	74.2 (71.7-80.7)	19.8 (15.2-23.3)

Legend: TOI - Tissue Oxygen Index, DropTOI was calculated as the difference between average baseline TOI and the lowest TOI. Characteristics are presented as median with interquartile ranges in brackets.

Figure 6.3: Variation in Baseline Tissue Oxygen Index in the First 5 days of Critical Illness

Serial baseline tissue oxygen index as measured by NIRS VOT. Blue line represents the mean baseline TOI. The red dashed line with triangles denotes upper confidence interval while the red dashed line with squares denotes lower confidence interval.

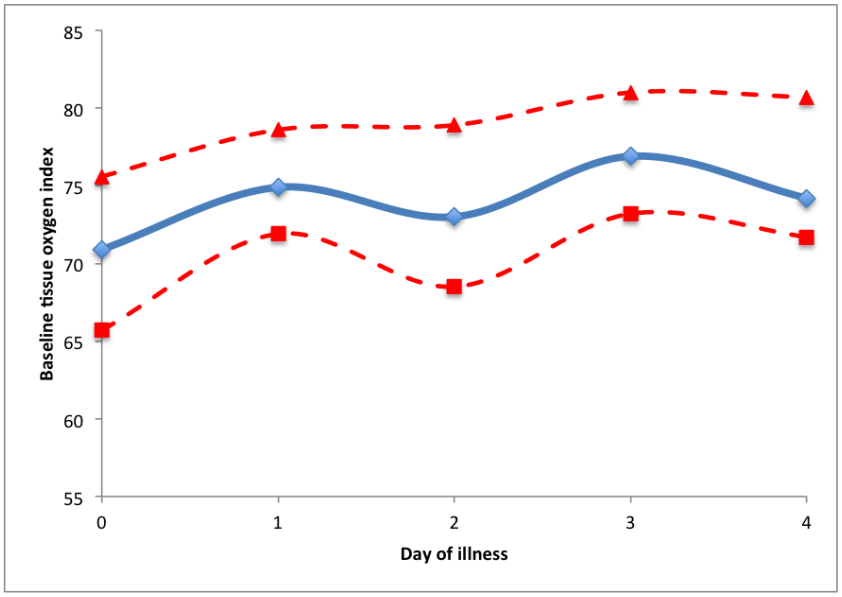
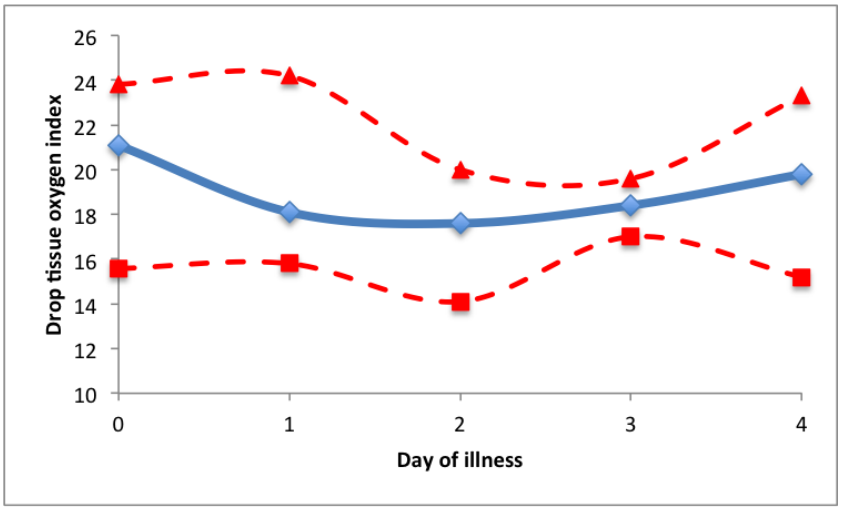


Figure 6.4: Variation in Drop Tissue Oxygen Index in the First 5 days of Critical Illness

Serial drop tissue oxygen index as measured by NIRS VOT. Blue line represents the mean drop TOI. The red dashed line with triangles denotes upper confidence interval while the red dashed line with squares denotes lower confidence interval.



Univariate analysis

The Drop TOI was the outcome measure for the univariate analyses. Age, weight, CaO_2, haemoglobin, baseline TOI and status at discharge had a significant association (Table 6.6). The day of illness, PELOD2 score, sex, diagnosis, cardiac index, PaO_2, SaO_2 and DO_2I did not have a statistically significant association. (Table 6.5)

Table 6.5: Results of univariate analysis - DropTOI as outcome

Co-variate	**Coefficient**	**Standard error**	**p value**
Age (months)	-0.02	0.01	0.03
Weight (Kgs)	-0.09	0.05	0.05
CaO_2 (ml/100ml)	-0.01	0.003	0.0001
Haemoglobin (gm/dl)	-1.13	0.31	0.0004
Baseline TOI	-0.24	0.11	0.03
Alive at ICU discharge	-7.43	1.94	0.0002

Key: CaO_2 - Arterial oxygen content, ICU - Intensive care unit, TOI - Tissue Oxygen Index

Multivariate analysis

The multilevel multivariate regression analysis displayed considerable variation in drop TOI in the first 5 days of illness. The day of illness (despite no association on univariate analysis) and variables that had an association on univariate analysis were chosen as co-variates for fixed effects in the final model. Weight was excluded as it has a co-linear relationship with age. CaO_2 was excluded. Only haemoglobin showed an association in univariate analysis of the three variables that constitute CaO_2. Drop TOI might predict the status at discharge as demonstrated by the

univariate analysis. But the relationship cannot be reversed. Therefore, this variable was also excluded. The final model shows that only haemoglobin remained a significant predictor of drop TOI (Table 6.6).

Table 6.6: Multilevel regression analysis - Drop TOI as outcome

Co-variate	**Beta-coefficient**	**Std.err**	**LCI**	**UCI**
Day 2	-0.30	1.37	-3.03	2.43
Day 3	-1.59	1.33	-4.27	1.05
Day 4	1.53	1.66	-1.75	4.87
Day 5	0.48	1.63	-2.80	3.72
Age (months)	-0.02	0.01	-0.05	0.008
Haemoglobin	-0.63	0.21	-1.07	-0.20
Baseline TOI	-0.08	0.09	-0.26	0.10

Key: Multilevel model with Drop Tissue Oxygen Index (TOI) as the outcome measure. The co-variates - day of illness, age, haemoglobin and baseline TOI - were nested within individuals, LCI - Lower confidence interval, UCI - Upper confidence interval.

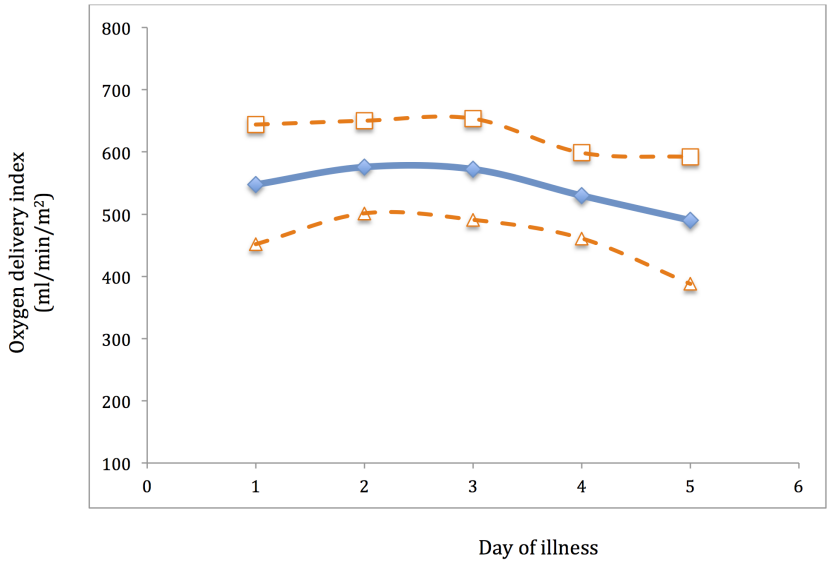
Oxygen delivery and consumption

The cardiac index did not vary during the first 5 days of illness. The USCOM

estimate of oxygen delivery also remained stable (Figure 6.5).

Figure 6.5: Variation in the mean USCOM estimate of oxygen delivery in the first 5 days of critical illness

The Ultrasound Cardiac Output Monitor (USCOM) estimate of oxygen delivery was calculated for all patients just prior to the near infrared spectroscopy vascular occlusion test and then the mean value represented as the blue line in the graph. Cardiac index was measured by the ultrasound cardiac monitor. The blue line represents the mean oxygen delivery. The Orange dotted lines represent upper and lower 95% confidence intervals.

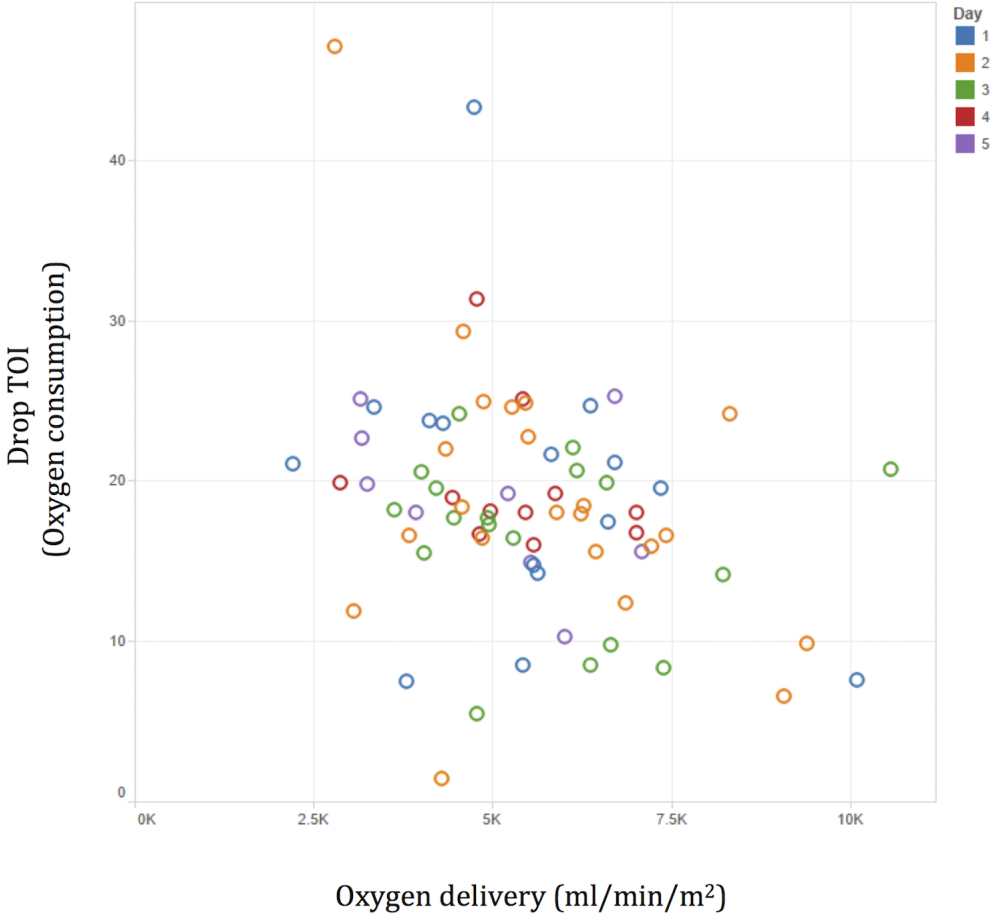


Legend: The oxygen delivery index was calculated for each patient just prior to the near infrared spectroscopy vascular occlusion test. Cardiac index was measured by Ultrasound cardiac monitor.

Oxygen consumption (Drop TOI) was plotted against oxygen delivery for the first 5 days of illness. The scatter plot suggests that there may not be an association between the two variables. This concurs with the univariate analysis.(Figure 6.6)

Figure 6.6: Relationship Between Drop Tissue Oxygen Index and Oxygen Delivery

The oxygen delivery was calculated for each patient for each recording of Drop Tissue Oxygen Index (TOI) (surrogate for oxygen consumption). The blue colored circles are values from day 0, orange circles are day 1 values, green circles are day 2 values, red circles are day 3 values and purple circles represent day 4 values.



Functional data analysis comparing NIRS VOT curves for each day of illness

The TOI data was converted into functional form as detailed in chapter 5 (section

5.7.1). The process was repeated for all the 94 patient NIRS VOT recordings in the first 5 days of illness (94 final functional curves from 35 patients). (Figure 6.7)

The 94 functional curves were combined (average of each time point for each day of illness) to plot predicted average functional curves. This resulted in five curves (one for each day from 0 to 4). (Figure 6.8)

Figure 6.7: Functional Data Analysis Curves of NIRS VOT in the First 5 days of Illness

Functional curves from first 5 days of intensive care stay are presented in this plot. The top panel shows curves on day 0 and 1. The middle panel depicts curves from day 2 and 3. The lower panel depicts day 4 curves. The recording starts with a baseline section. This is followed by the downslope on inflation of the blood pressure cuff at 302.5 seconds. The cuff is deflated at 461.5 seconds causing the upslope. The final section is the recovery phase.

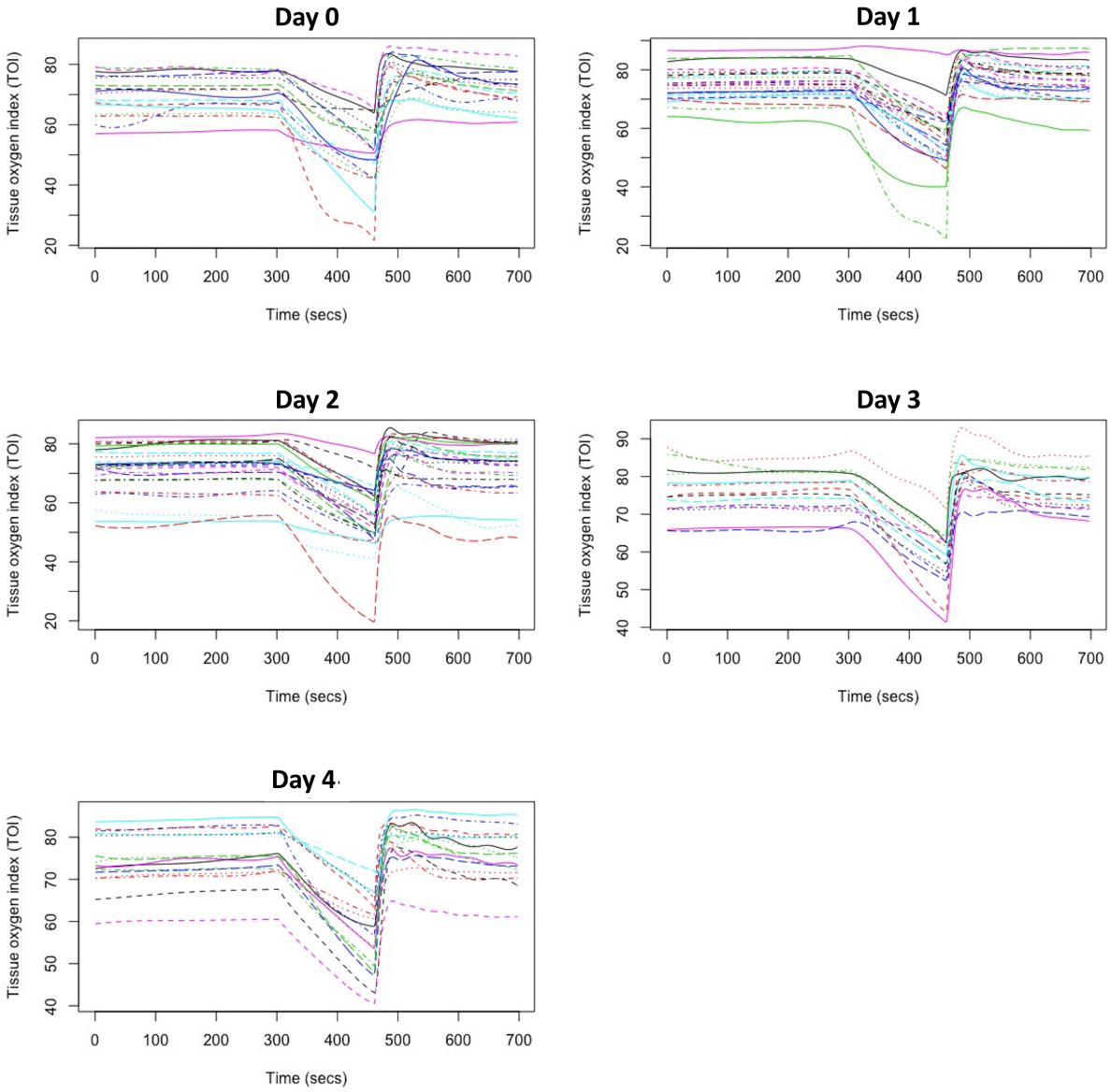
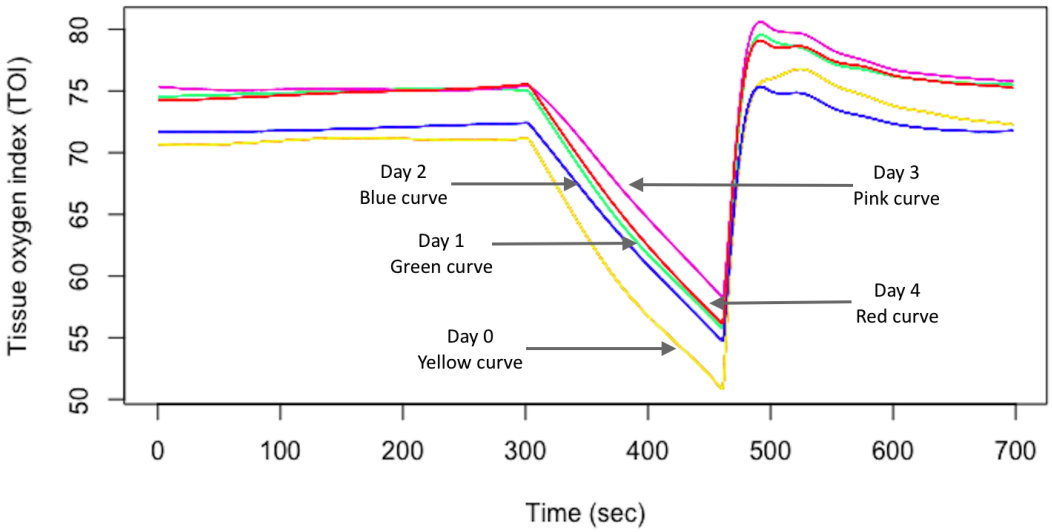


Figure 6.8: Average Curves for Each day of Illness Predicted Individual Functional Curves

Yellow curve - Day 0, Green curve - Day 1, Blue curve - Day 2, Pink curve - Day 3, Red curve - Day 4. The predicted curves were created by using all the functional curves for recordings in the intensive care unit for the day of illness from 0 to 4.



The functional curves enabled the comparison of the patient NIRS VOT recordings in two ways. The first was with a multilevel functional regression analysis and the second was with a functional ANOVA. The multilevel functional regression method was used in this dataset (not used in YES2) as there were several missing data points for each individual patient as depicted in figure 6.1.

If a functional ANOVA had been employed, the predicted curves for days 1 to 4 would have been individually compared (whole curve) to day 0 curve. This was

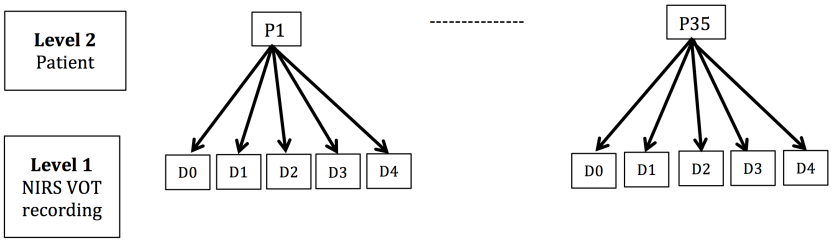
different to the YES2 data analysis due to missing data points. Of note, using the predicted curves, rather than individual curves, would result in loss of information (unmatched data). Therefore, the multilevel model with functional data was chosen as the ideal analysis for this dataset.

Multilevel functional regression analysis - Tissue Oxygen Index vs Day of illness

The individual functional curves generated (94) were utilised for the multilevel regression model. The TOI was the outcome measure. The individual patient was placed at level 2) similar to the YES2 data analysis under section 5.6.3. (Figure 6.9) The day of illness was the co-variate.

Figure 6.9: Multilevel Model for Serial NIRS VOT

Organisation of the levels for analysis of serial NIRS VOT data



From the final model, values for the sections of the NIRS VOT curve were calculated. The code used by the FDA mixed package to generate these values is detailed in the appendix. The beta-coefficient for day of illness was -0.34 (95%CI: -1.37,

0.69, p = 0.31). The table 6.7 enumerates the values. The first row shows the baseline, lowest and drop TOI on day 0. The next four rows note the difference in TOI values compared to day 0.

The day of illness had no detectable influence on the shape of the whole NIRS VOT curve. However, day of illness was associated with differences in the observed drop TOI with each of days 1-4 being significantly lower than on day 0 in the multiple level regression analysis.The drop TOI was calculated as the difference between the baseline TOI and the lowest TOI. The variation in drop TOI might suggest that oxygen consumption changes during recovery from critical illness. Figure 6.11 was plotted to visualise this variation in drop TOI. No confidence interval has been reported for the FDA generated drop TOI values. I was unable to calculate the interval due to limitations of the software package employed in the R software.

Table 6.7: Multilevel functional regression of the near infrared spectroscopy with vascular occlusion test curve

Fixed effect	**Baseline TOI (95%CI)**	**Lowest TOI (95%CI)**	**Drop TOI**	**p-value**
Day 0	70.88	50.89	19.99	
Day 1	4.02 (3.70-4.33)	5.06 (4.74-5.37)	- 1.04	<0.0001
Day 2	1.45 (1.13-1.76)	4.02 (3.70-4.33)	- 2.57	<0.0001
Day 3	4.61 (4.29-4.92)	7.63 (7.31-7.94)	-3.02	<0.0001
Day 4	4.63 (4.31-4.94)	5.56 (5.24-5.87)	-0.93	<0.0001

Legend: Multilevel model with the whole near infrared spectroscopy curve as outcome. The day of illness was included as the fixed effect co-variate. The day of illness was normalised for day 0. The values of the baseline Tissue Oxygen Index (TOI) and lowest TOI for day 1 to day 4 are the difference when compared to day 0 values. The values in brackets denote 95% confidence interval. The drop TOI value is the calculated difference between baseline TOI and lowest TOI. No confidence interval has been shown for drop TOI due to inability to calculate the interval with a restriction within the R software.

Figure 6.10: Functional Data Analysis Derived Relationship Between Oxygen Consumption and Day of Illness

Functional data analysis (FDA) was employed to calculate the change in oxygen consumption. Functional curves were created for all 94 recordings of near infrared spectroscopy with a vascular occlusion test. A multilevel FDA was used to compare functional curves from each day of illness. The values of Drop Tissue Oxygen Index (DropTOI) was calculated from the model and plotted against day of illness. No confidence interval has been shown due to inability to calculate the interval with a restriction within the R software.

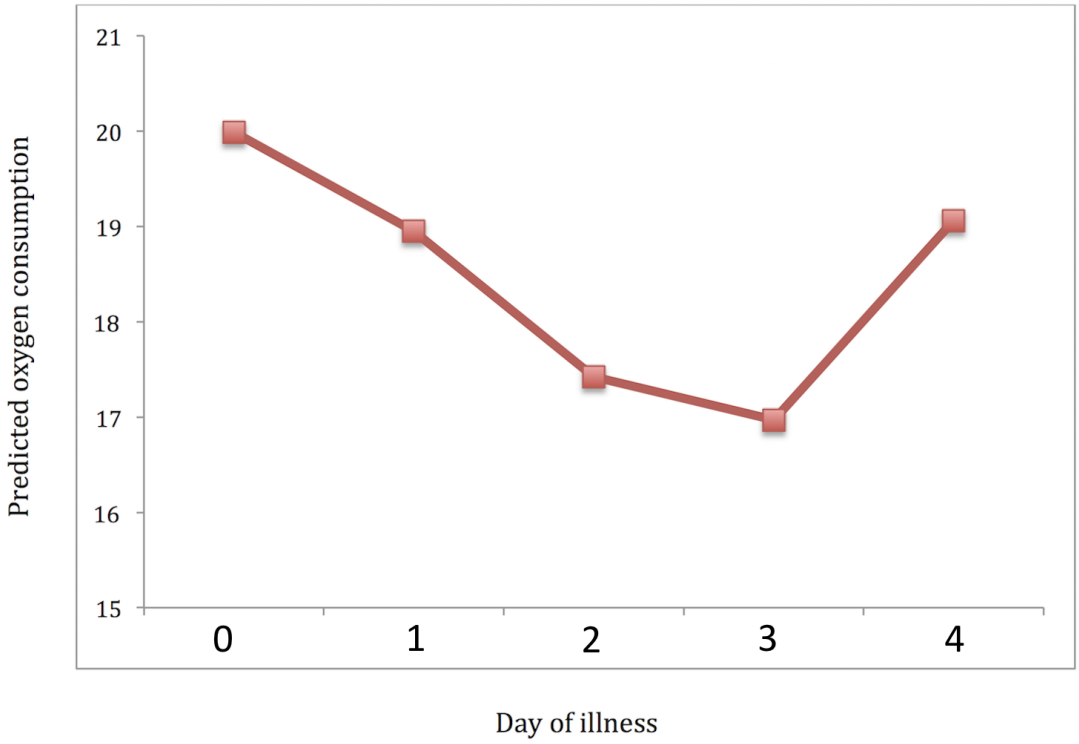
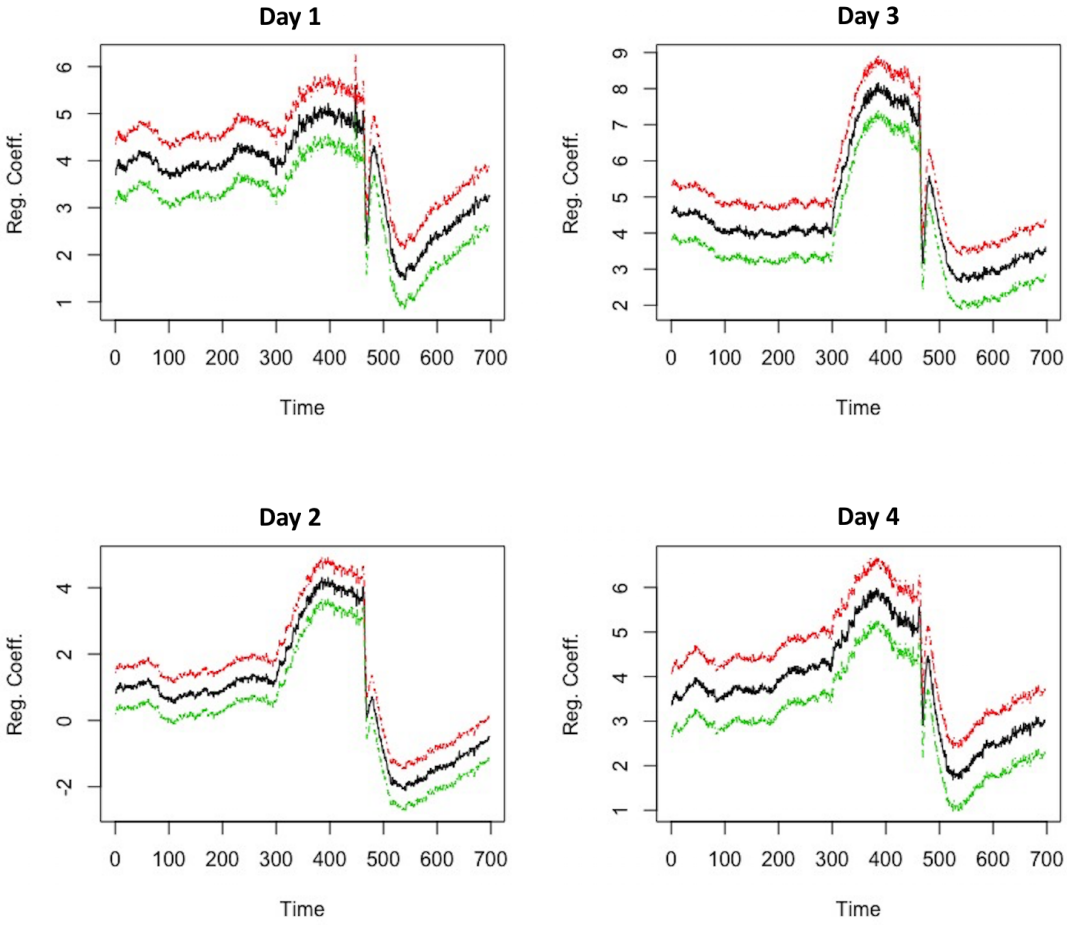


Figure 6.11 shows the regression coefficient curves with upper and lower confidence intervals estimated by the mutlilevel model. All 4 curves were statistically different to day 0 curve as demonstrated in table 6.7.

Figure 6.11: Functional Regression with Multilevel Model - Day 1-4 vs. Day 0

Regression coefficient generated with multilevel model comparing day 1-4 with day 0. Blue curve - mean regression curve, Red curve - upper confidence interval, Green curve - lower confidence interval



To summarize, FDA of NIRS VOT in critically ill children in the first 5 days shows that forearm dropTOI decreases with duration of illness. Interestingly, the drop TOI does not revert to day 0 values on day 4 (Figure 6.10). The normal pre-admission / pre-illness drop TOI value for the ICU patients is not known.

Therefore, it is unclear which if the two values if any - day 0 or day 4 drop TOI are equal to normal. The only certain interpretation is that there is variability in the drop TOI values with duration of illness.

6.4.2 The O2K study

The flowchart depicts the number of children recruited in the study.(Figure 6.12) Data from 8 patients was used for the final analysis. The median age and weight were 4.12 years and 15.5 kgs respectively. There were an equal number of boys and girls in the group. A majority of the recruited children were admitted following sepsis associated with systemic inflammatory response syndrome. (Table 6.8) The Figure 6.13 notes the timing and number of repeats for each patient.

Figure 6.12: Flowchart of Recruits in the O2K Study

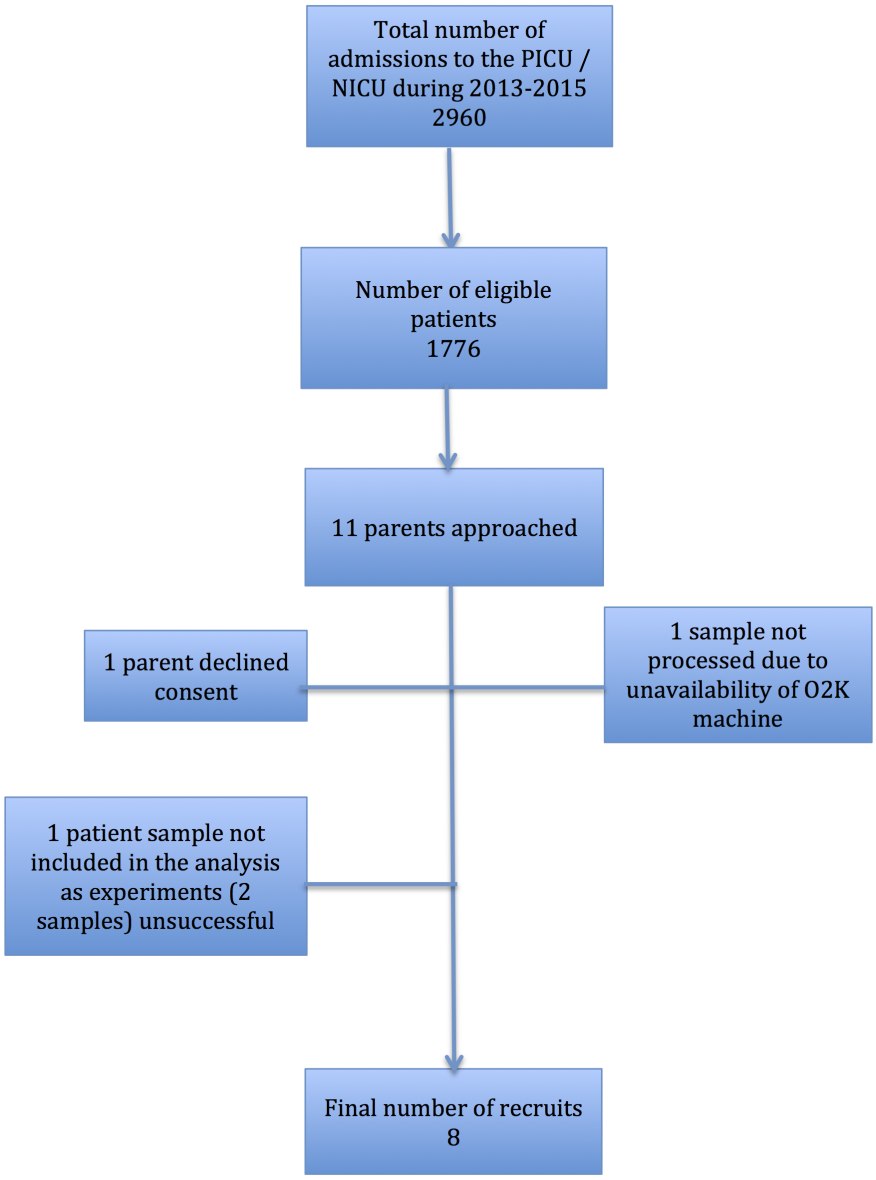


Figure 6.13: Timing and Number of Repeats of O2K Analysis

ID	Day 0	Day 1	Day 2	Day 3	Day 4
SOCK101	x	x			
SOCK102	x	x	x		x
SOCK103		x		x	x
SOCK104		x	x		
SOCK105	x	x	x	x	x
SOCK107		x	x		x
SOCK108	x	x	x	x	
SOCK109	x	x		x	

Recordings from patient id SOCK106 were excluded due to unstable trace.

Table 6.8: Details of children recruited to serial O2K study

Characteristic	**Value, n = 8**
Age (years)	4.12 (IQR:1.26-7.47)
Weight (Kgs)	15.5 (IQR:10.0-22.7)
Cardiac index ($L/min/m^2$)	3.9 (IQR:3.8-4.6)
Sex	
Male	4 (50%)
Female	4 (50%)
Diagnosis	
Respiratory	2 (25%)
Sepsis	6 (75%)
PELOD2 (range)	4-12
Alive at ICU discharge	7 (87.5%)

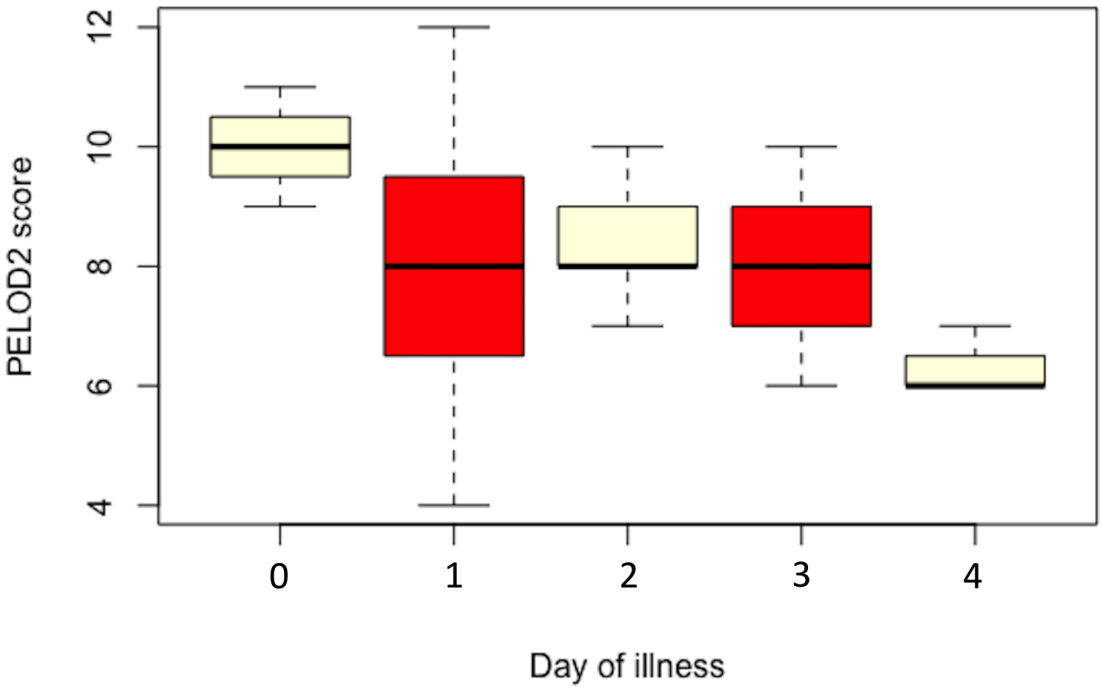
Legend: Characteristics are presented as median with interquartile range (IQR) or absolute number with percentages. PELOD2 - Pediatric logistic organ dysfunction score 2, ICU - Intensive care unit. PELOD2 was calculated with each episode of blood draw for the O2K analysis.

PELOD2

The median PELOD2 score decreased with duration of illness. (Figure 6.14) It ranged between 4 and 12.

Figure 6.14: Distribution of Pediatric Logistic Organ Dysfunction 2 score (PELOD2) in the First 5 days of Illness

Box plot of the variation in the PELOD2 score. The bold horizontal line within the box plots represents median values. The box represents the interquartile range. The error bars denote the range of values.



Raw O2K measurements

Table 6.9 shows summative raw measurements from the O2K experiments. The values were measured in $picomols.sec^{-1}.10^{-6}cells$. There are two inherent prob-

lems with these values. First, the measurements are not normalised for the individual patient. Second, the data were not normalised for the mitochondrial content, as this variable was not measured. Consequently, the oxygen consumption might seem to vary between the days and between the patients. Therefore, utilising the crude measurements for the final analysis would not be ideal. Instead, I have employed the parameters that are independent of mitochondrial number (the flux control ratios). These were decreibed in detail in chapter 2 (section 2.3.3).

Table 6.9: The crude O2K measurements

IQR - Interquartile range, ETS - Electron Transport System capacity, ROX - Residual oxygen consumption. The values were measured in $picomols.sec^{-1}.10^{-6}cells$

Day of illness	**Routine respiration (IQR)**	**Leak respiration**	**ETS (IQR)**	**ROX**
Day 0	3.7 (2.9-4.5)	1.4 (0.8-1.9)	4.1 (3.1-5.6)	0.5 (0.4-0.7)
Day 1	6.6 (4.8-7.9)	3.2 (1.2-5.9)	8.9 (7.6-9.6)	1.2 (0.2-3.0)
Day 2	9.9 (4.5-11.6)	7.6 (2.1-9.1)	12.9 (9.4-16.8)	4.9 (0.8-6.4)
Day 3	6.8 (4.7-10.5)	3.7 (0.9-8.3)	8.7 (8.1-12.6)	1.9 (0.3-4.9)
Day 4	15.8 (7.8-22.8)	11.1 (3.8-18.3)	20.3 (13.1-26.4)	6.8 (2.0-11.9)

Flux Control Ratio results

Net Routine Control Ratio

The Net RCR dropped from day 0 to day 4. Figure 6.15 suggests that the drop was more marked in the fist 24 hours of illness. The plot is a summative measure and does not account for the multiple levels in the data. Therefore, a multilevel model analysis was performed.

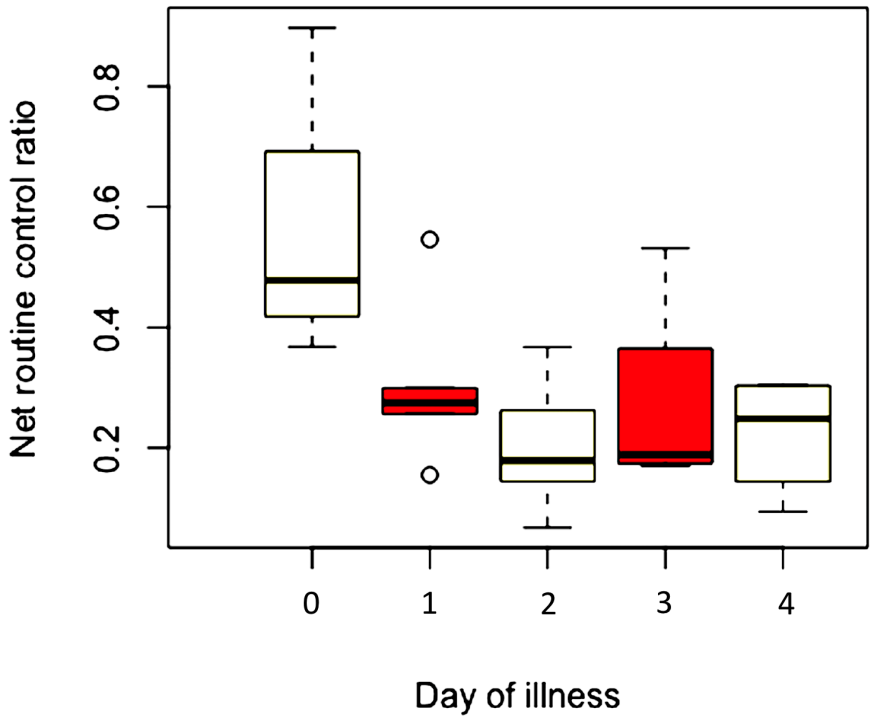
The association between Net RCR and day of illness was analysed in a multilevel regression model. The Net RCR decreased by 0.06 units (95%CI: -0.09, -0.03) for each additional day of illness compared to day 0 (Figure 6.11). Net RCR [(R-L)/E] is the difference between routine respiration (R) and leak respiration (L) as a proportion of maximal stimulated respiration (E). The maximal stimulated respiration is note expected to change during critical illness. Consequently, the drop in Net RCR has to be either due to a decrease in routine respiration or an increase in leak respiration. The decrease in routine respiration during recovery from critical illness might be due to reduction in the basal metabolic rate and conversion to a anabolic state. Net RCR might be artificially high on day 0 due to excessive ATP demand. An increase in leak respiration is unlikely in this scenario

as the LCR decreases as detailed later.

If hypoxic adaptation were to occur, then one would expect a rise in Net RCR. Therefore, the Net RCR results do not seem to support the hypothesis of hypoxic adaptation. But they suggest the patient may have reached a steady state by day 1. On inspection, the figure 6.15 shows that Net RCR drops until day 2 and then starts to rise again. It is possible that the change in Net RCR is monotonic after day 2. Irrespective of the type of change (monotonic or non-monotonic), this has not been captured in the multilevel model. If the measurements were continued for a longer duration, there may be an increase in Net RCR. However, within the remit of this project I was unable to investigate this process.

Figure 6.15: Variation of Net routine control ratio in peripheral blood mononuclear cells estimated by the Oroboros analyser in the first 5 days of illness

Box plot of the variation in the net routine control ratio. The bold horizontal line within the box plots represents mean values. The whiskers denote 95% confidence intervals.



Leak Control Ratio

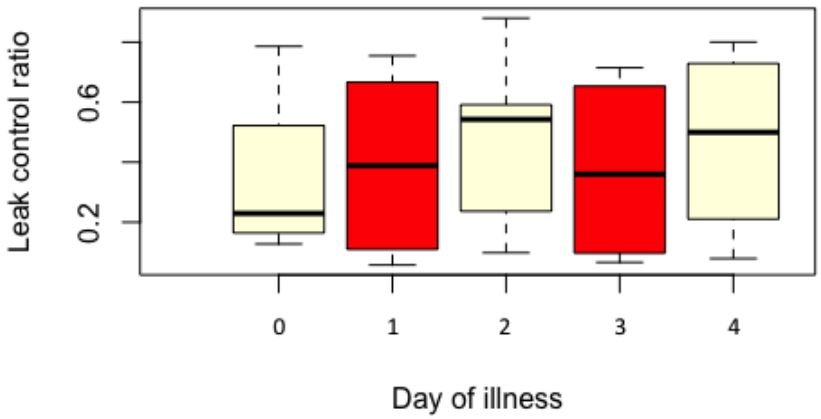
The Leak Control Ratio (LCR) plot may indicate an increase in value from day 0 to day 4. (Figure 6.16) Similar to the Net RCR plot, this utilised a summative measure and does not account for the multiple levels in the data. Therefore, a multilevel model analysis was performed.

The LCR dropped by 0.05 units (95%CI: -0.07, -0.03) for each additional day of

illness. The LCR (L/E) is the leak respiration (L) as a proportion of the maximal stimulated oxygen consumption (E). This drop in LCR suggests decrease in uncoupling and an increase in coupling state. Therefore, the electron transport chain function may have become more efficient. This result is in keeping with the hypothesis of hypoxic adaptation.

Figure 6.16: Variation of Leak Control Ratio in Peripheral Blood Mononuclear Cells Estimated by the Oroboros Analyser in the First 5 days of Illness

Box plot of the variation in the leak control ratio. The bold horizontal line within the box plots represents mean values. The error bars denote 95% confidence intervals.



Coupling efficiency

Coupling efficiency [(E-L)/E] is the difference between maximal stimulated oxygen consumption (E) and leak respiration (L) as a proportion of maximal stimulated

oxygen consumption (E). The mean coupling efficiency increased marginally from day 0 to day 4. However, there were considerable individual variations and a wide range of values (<0.1 to >0.9). (Figure 6.14 and 6.15) The results might suggest that from day 0 to day 4 the amount of coupling increased. This supports the hypothesis of hypoxic adaptation.

Figure 6.17: Coupling Efficiency from O2K Analysis - Mean Values

The plot represents mean data for 8 children over the 5 days of analysis. The coupling efficiency was calculated by subtracting the leak respiration from Electron Transport System capacity (ETS) and dividing by ETS. It was normalised for the first recording of the patient. The mean values were plotted with 95% upper and lower confidence intervals.

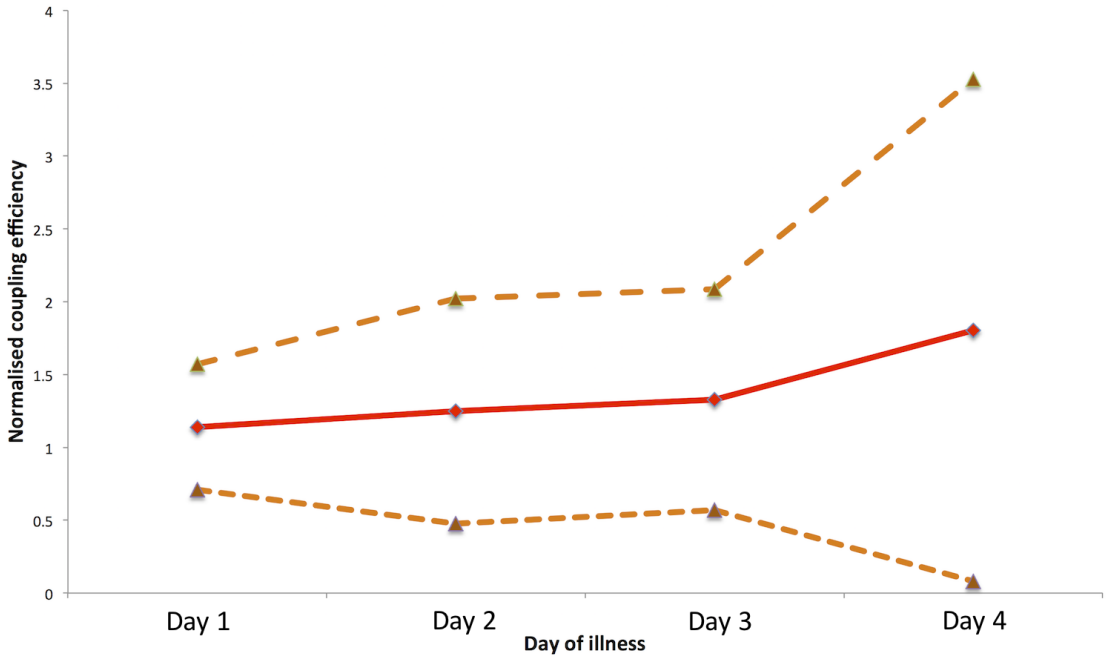
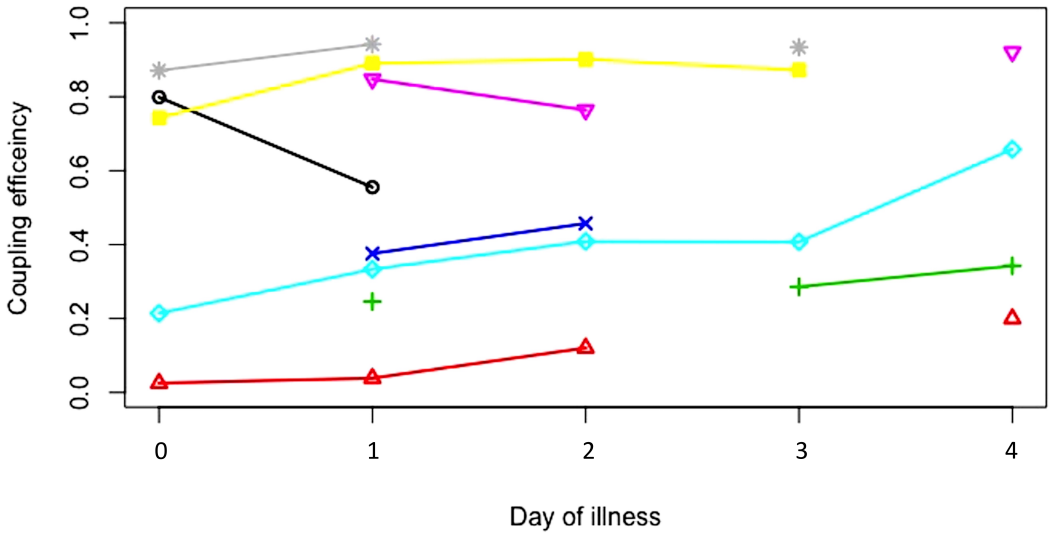


Figure 6.18: Coupling Efficiency from O2K Analysis

The colors represent individual patient data. The coupling efficiency was calculated by subtracting the leak respiration from Electron Transport System capacity (ETS) and dividing by ETS.



Times series comparison of O2k and NIRS VOT analysis

The NIRS VOT measurement of the recruits in the O2K study is detailed in table 6.10. The median DropTOI varies from day 0 to day 4.

Table 6.10: Near infrared spectroscopy with a vascular occlusion test measurements of the O2K study recruits

TOI - Tissue oxygen index, Median values with interquartile ranges are presented.

Day of illness	**Baseline TOI**	**Lowest TOI**	**Drop TOI**
Day 0	64.5 (61.5-70.4)	45.1 (41.3-54.9)	15.1 (12.9-18.5)
Day 1	78.9 (73.6-80.6)	57.4 (45.9-61.8)	21.5 (17.9-27.9)
Day 2	75.0(69.2-77.4)	44.7 (40.3-57.5)	29.7 (14.5-32.7)
Day 3	71.0 (70.4-71.2)	52.4 (43.1-59.6)	17.8 (9.7-28.4)
Day 4	75.6 (72.4-79)	56.8 (53.7-62.1)	18.3 (14.6-20.6)

The comparison of the NIRS VOT measurements and the O2K results was performed in two steps. First, a scatter plot of Drop TOI and Net RCR was created. (Figure 6.19) This suggested that there was no correlation between the two values. Next, Spearman correlation analysis was performed. In order to account for the multiple data points for the same patient, flux control ratios were normalised for the first value of the patient.

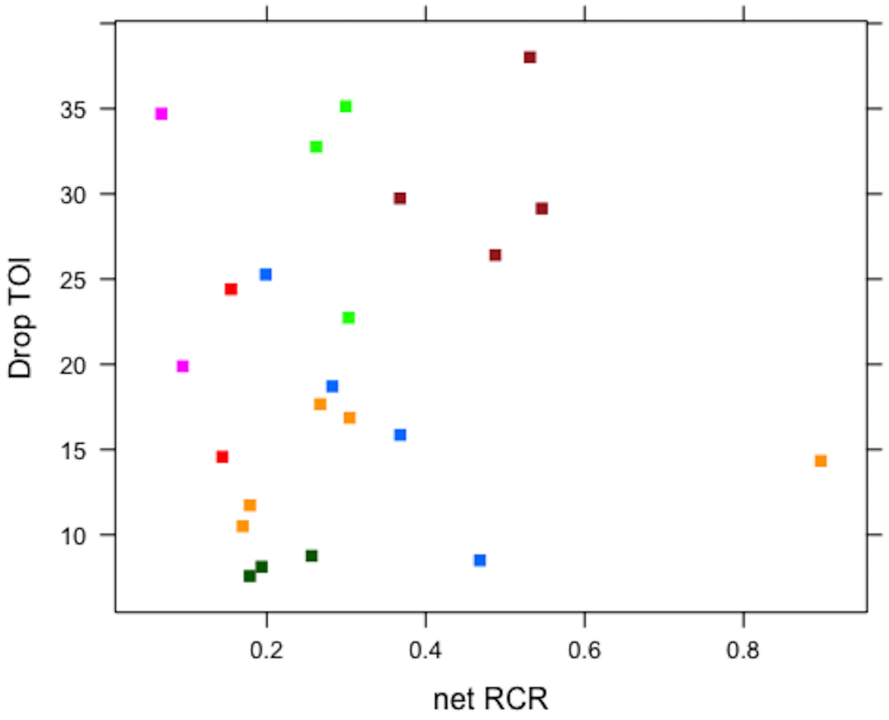
Following this, the Drop TOI was compared to all three flux control ratios. None of the pairs demonstrated a correlation (Drop TOI with normalised Net RCR , p =0.09; Drop TOI with normalised LCR, p = 0.7; Drop TOI with coupling effi-

ciency, p =0.9).

The interpretation of this analysis was that the two techniques assess different tissues in different ways. Therefore, a comparison is not the ideal mode of analysis.

Figure 6.19: Scatter Plot of the Drop Tissue Oxygen Index (TOI) and net Routine Control Ratio (net RCR)

The colours represent different patients.



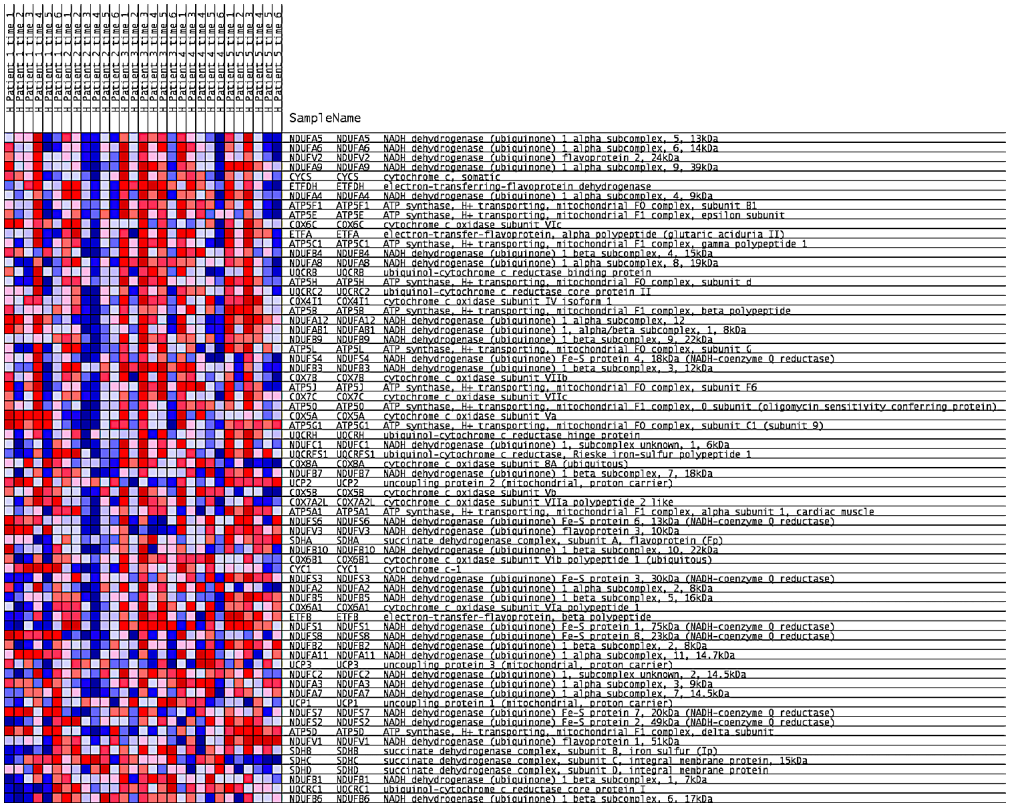
6.4.3 The OXPHOS Gene Expression Study

The GSEA ranks changes in single gene expression. The focus was on the Reactome TCA cycle and Respiratory Electron Transport Chain (RETC) genes within the 3655 included gene sets.

Figure 6.17 shows the heat map of the gene expression profile ordered as a time series. On the group level analysis, with a False discovery rate (FDR) at <25% and ranked according to their Normalised Enrichment Score (NES), 1920 out of 3655 gene sets showed a decreasing profile with time. The RETC set was ranked 75th of the 1920 gene sets.(Figure 6.18)

Figure 6.20: Heat map of the Gene Expression

Heat map of the gene expression profile over the first 48 hours measured at 6 time points in 5 patients.



On the individual level analysis and FDR <25%, 3 patients had a highly ranked fall in RETC set expression. Patient 1 showed a low correlation of RETC gene

expression to a decreasing profile. This might be relevant as, unfortunately, this child died. Atleast half of the gene sets of the RETC gene set had a positive correlation with the class file (decreasing profile in my analysis) in all 5 patients.(Table 6.11)

Table 6.11: Normalised enrichment score, FDR and nominal p values for the RETC gene set

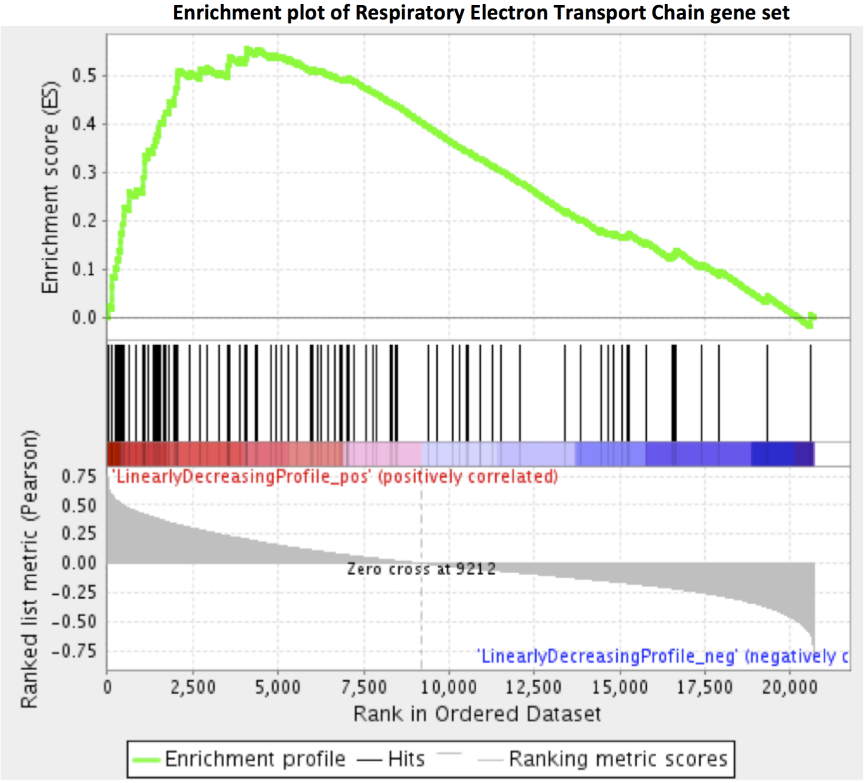
NES - Normalised Enrichment Score, FDR - False Discovery Rate, RETC - Respiratory Electron Transport Chain gene set

Patient	**NES**	**Nominal p-value**	**FDR**	**Position of RETC**	**Number with positive correlation**
1	0.932	0.598	0.710	1648	2017
2	1.006	0.462	0.573	1610	2108
3	1.971	<0.001	0.008	124	1895
4	2.787	<0.001	0	51	1819
5	3.015	<0.001	0	5	2036

The GSEA team (Broad institute, MIT) helped with the analysis of these results. I analysed the gene expression using GSEA and for external validation contacted the Broad institute team. They independently analysed and concurred my results.

Figure 6.21: Enrichment Plot of Respiratory Electron Transport Chain Gene Set

Gene Set Enrichment Analysis (GSEA) compares the variation in the gene expression in our overall dataset compared to a preselected gene set (Respiratory Electron Transport Chain gene set) over the time course. The RETC geneset expression is downregulated more than would be expected by background variation in expression. The top portion of the plot shows the running enrichment score (ES) for the RETC gene set. The middle portion of the plot shows where the members of the gene set appear in the ranked list of genes. The lower portion of the plot shows the value of the ranking metric as you move down the list of ranked genes. The ranking metric measures a genes correlation with a phenotype. For my continuous phenotype (time series), a positive value indicates correlation with the phenotype profile (decreasing profile). The table shows the Normalised Enrichment Score (NES), False Discovery Rate (FDR) and nominal p values for the RETC gene set.



Patient	NES	Nominal p value	FDR	RETC position	Gene sets with decreasing expression
Group	2.55	**<0.001**	0	75	1920

6.5 Conclusions

The oxygen consumption shows a pattern of variation similar to that described in adults. [108] The VO_2 is high on day 0 of admission to intensive care. By day 3, it has reached a nadir. Following this, VO_2 increases. The levels reached on day 4 are still lower than that on day 0. This may be in keeping with the primary hypothesis of my thesis i.e. hypoxic adaptation occurs during recovery from critical illness. The variation in VO_2 is unrelated to changes in DO_2. As TOI is calculated as a ratio of oxygenated to total haemoglobin, the association noted between haemoglobin and DropTOI in the multilevel model is unsurprising.

The PELOD2 score drops from day 0 to day 4. There are two points to consider here:

1. The decrease in PELOD2 score suggests recovery from critical illness. One might hypothesise that the basal metabolic rate is also normalising during this phase. How might this affect the oxygen consumption from the peripheral blood mononuclear cells? There are several confounders - such as mechanical ventilation, drugs, feeds etc - that preclude a simple answer. But with a bigger sample size, these could be accounted for and further interpretation might be possible.
2. If hypoxic adaptation were a mode of recovery from critical illness, then the phe-

notype would be a drop in oxygen consumption as the PELOD2 score decreases. As suggested earlier, a larger sample with regular measurements for each patient might explain these issues.

The serial NIRS VOT findings have to be interpreted with caution as different children were studied at different time points making this a potential confounder.

The NetRCR drops with increasing duration of illness. This suggests that the increased ATP synthesis needed in the first 24 hours of illness might be normalising by day 2. No firm conclusion can be drawn without further study with a larger sample size. However, my study lends itself as proof of concept. It is feasible to measure oxygen consumption from peripheral blood mononuclear cells in critically ill children.

Mitochondrial function alters during critical illness, but the extent to which this is adaptive or harmful is unclear. [314] Mitochondrial function changes and fatty acid oxidation increases with protracted critical illness. This is in the presence of insulin and glucose. [173] Recently, hypoxia has been used as therapy in mitochondrial disease in an animal model. [246] Reduced mitochondrial oxygen utilisation and gene expression have been observed in established sepsis in adults and children. [317] Following an intravenous administration of endotoxin dose, Calvano et

al profiled gene expression in white blood cells in 8 adult volunteers at predetermined time intervals. By using a genome-wide network analysis, they demonstrate a transient bioenergetics failure in the white blood cells in these volunteers. [316] My OXPHOS gene expression analysis concurs with these reports. The downregulation of OXPHOS genes fits with the theory of mitochondrial dysfunction in the initial phase of critical illness. Unfortunately, I did not have the gene expression profile for the later half of intensive care stay.

I hypothesise that perhaps the relative increase in drop TOI seen on day 5 might be secondary to the upregulation of OXPHOS genes. However, only a more extended gene expression study will be able to investigate this theory further.

It is probably simplistic to correlate gene expression to oxygen consumption. In addition, the clinical implication of my gene expression finding is difficult to extrapolate from the small sample size. Notwithstanding, my study is suggestive of decreased oxygen consumption in the first 48 hours of critical illness.

Limitations of O2k technique

The main limitation of the O2K project was its small sample size. This severely restricts the ability to extrapolate findings. One of the strengths of the O2K analyser is that it can measure oxygen consumption from permeabilized tissues. This is also a limitation as there is an extrapolation of the measured oxygen consumption

from cells to the whole body.

The L/E ratio can increase if ETS capacity reduces. The abnormalities in ETS capacity can be investigated by comparing it to citrate synthase activity. I did not undertake this part of the experiment and would like to include this analysis in future work. [327]

Another parameter that can be a limitation is the vasomotor tone. The vasomotor tone of the critically ill patient can be labile especially in the first 48 hours of illness. This might create variation in oxygen delivery to the tissues. Further, the effect of intensive care management strategies such as mechanical ventilation, use of inotropes, mode of nutrition, blood transfusion and fluid resuscitation may have an effect on the analysis. Mode of nutrition becomes relevant as blood glucose concentration affects oxygen consumption by mitochondria. [328]

There is no correlation between measures of oxygen consumption by NIRS VOT and O2K. Even if there were to be an association, the relationship would need to be analysed with caution as the measurements are from different tissues and different techniques.

Limitations of NIRS VOT

Ambient and peripheral skin temperature can have a major effect on the NIRS VOT measurements. [291] I measured the temperature of the child in the intensive

care at the time of the NIRS VOT recording. This value was then incorporated into the regression model as one of the co-variates.

NIRO-NX 200 uses multichannel measurements to reduce regional variations in skeletal muscle oxygenation. This is pertinent for the serial measures of TOI in the intensive care patients. Despite the possibility of probe placement at different sites, I have demonstrated reproducible and comparable TOI values.

6.5.1 Do my Results Support the Hypoxic Adaptation Hypothesis?

With the serial NIRS VOT study, I have demonstrated that the oxygen consumption from forearm muscle drops until day 3. Most importantly, day 4 values are lower than day 0 values. This suggests reduced oxygen consumption that might suggest a more efficient mitochondrial function. One hypothesis could be that mitochondria are able to produce the required ATP with a lower amount of oxygen consumption due to hypoxic adaptation.

The key result from the O2K study is the drop in leak control ratio with duration of illness. This drop may be due to a decrease in uncoupled respiration in favour of

a more coupled state. If this result were shown to be reproducible, then it would support the hypothesis of hypoxic adaptation.

The OXPHOS gene expression study showed a decrease in gene expression up to 48 hours of onset of sepsis associated critical illness. This is one part of the story for hypoxic adaptation. Ideally, gene expression profiles should be extended for a longer duration to observe if there is normalisation of expression. Further, a functional correlate is needed to assess recovery and relative level (compared to healthy controls) of mitochondrial function.

In summary, my projects have provided some insights that are suggestive of hypoxic adaptation.

Chapter 7

Conclusions and Future Plan

7.1 Cohort Study

From my national survey, it is evident that the oxygen delivery practices in paediatric intensive care units in the UK vary widely. The retrospective observational study showed that hypoxia is not a good predictor of outcome. Furthermore, there is a 'U' shaped relationship between PaO_2 and mortality in critically ill chidlren. The systematic review shows that the evidence for harm with hyperoxia is equivocal.

My observational study also suggests that the coeficient of PaO_2 in the hyperoxic range in the paediatric index of mortality 2 model may not be accurate. I would like to work with the national paediatric intensive care audit network (PCIANet)

to explore this further in the future.

My study was on data from a single centre over a 10-year period. The PICANet database holds data from all paediatric intensive care units across the UK from November 2002 to present date. The first step would be to use the significantly larger dataset to ascertain if the 'U' shaped relationship I observed in my study is reproducible. As a second step, I would like to investigate if this relationship persists after case-mix adjustment and accounting for confounders. Finally, if step one and two were successful, I would like to explore the possibility of changing the FiO_2/PaO_2 coefficient in the PIM2 model. My theory is that there needs to be three coefficients for FiO_2/PaO_2; one for the hypoxic range, one for the normoxic range and one for the hyperoxic range. This I believe will make the PIM2 model robust. To achieve this, I will need the statistical support and expertise of the PICANet team.

7.2 Children with Suspected Mitochondrial Disease

Children with mitochondrial diseases are a heterogeneous group. In keeping with this, the NIRS VOT does not show a clearcut threshold for diagnosis of this disease. However, the real niche of the NIRS VOT technique is perhaps in the prognosis

of the disease and as part of the outcome scale assessment tool. The utility in monitoring response to treatment strategies such as riboflavin supplementation needs further research. It would be prudent to validate the technique in another clinic on children with suspected mitochondrial disease before their muscle biopsy. Despite the limitations of the patient profile, NIRS VOT seems to be able to reflect oxygen consumption from forearm muscle.

7.3 Young Everest Study 2

The YES2 study suggests that it might be safe for children between 8-16 years to trek to an altitude of 3500 metres with a gradual ascent profile. The forearm oxygen consumption did not show a statistically significant difference under hypobaric hypoxic conditions when compared to sea level values. However, there seemed to be a trend towards a decrease. This finding needs to be repeated in another study.

Future work should include a larger sample size, longitudinal studies, additional altitudes, younger age groups and comparisons of oxygen consumption of healthy lowland children to indigenous Sherpa children. To further confirm my NIRS VOT findings, I would like to compare the NIRS VOT results with VO_2 max measurements measured at both altitudes from a formal cardiovascular exercise testing. Another member of the YES2 team has performed these tests. I would like to

compare my results with her's when they are available.

If invasive testing were possible, Oroboros-2k analysis of peripheral blood mononuclear cells and muscle fibres might allow better characterisation of the mitochondrial function in this population.

7.4 Children with Critical Illness

Critically ill children are a heterogeneous population. The etiology of admission to the intensive care unit can be varied such as infection, trauma, and post-operative recovery. However, the process of recovery once systemic inflammatory response (SIRS) sets-in is probably similar for all children. Due to the limitations of NIRS VOT, the sequential, parallel measurement of oxygen extraction with NIRS and the Oxygraph-2K (O2K) analyser was performed. These two measurements cannot be compared as they interrogate different tissues. However, the O2K experiments showed that these were feasible in this population of critically ill children.

A larger dataset with a more varied patient profile may well help distinguish the adaptive physiological response that occurs during resolution of critical illness.

The methods employed in my projects on critically ill children have shown favourable results. Therefore, I would like to extend these studies as follows:

1. Serial oxygen consumption The key would be to find a technique that gives reproducible results. Although NIRS VOT seems promising, ideally I would like to employ a method such as indirect colorimetry to measure serial oxygen consumption. All patients admitted to PICU who are expected to be ventilated for at least 48 hours would fit the inclusion criteria. The data should be captured everyday until day 7 or until hospital discharge (which ever is longer).

In addition to this, I would measure the oxygen delivery continuously whilst the oxygen consumption is measured. This will need a gold standard, dynamic cardiac output monitor. The best available technique for this might be echocardiography.

Finally, a measure of reactive oxygen species levels is mandatory. This will ascertain that hypoxic adaptation is not harmful to the patient in the short and long term.

2. In tandem with whole body oxygen consumption, I want to measure consumption from several tissues. The O2K is a good method to perform this investigation. First, I want to perform oxygen consumption measurements from peripheral blood mononuclear cells in cyanotic congenital heart disease children and matched acyanotic congenital heart disease children. This might highlight the duration of hypoxic exposure needed for hypoxic adaptation to occur in critically ill patients.

Next, I would measure oxygen consumption from several tissues such as peripheral

blood mononuclear cells, muscle fibres, skin and cardiac muscle (from those undergoing cardiac surgery). This will answer two questions: a) How to account for mitochondrial heterogeneity? and b) Is there a linear relationship between oxygen delivery and consumption at the tissue level?

All the studies on O2k will need to have a measure of mitochondrial content (perhaps a measure of Cardiolipin as a surrogate marker).

On the general PICU, I would like to recruit a larger sample of patients who maybe ventilated for longer than 2 days.

3. To understand the pathophysiological basis of adaptation at the cellular level, I would like to investigate the HIF pathways as part of future studies in critically ill children.

4. The last part of the project would be to assess gene expression. I would focus on EPAS1 and OXPHOS genes. The expression profile would need to be extended to about 7 days.

Another project would be to review genes that are up regulated in hypoxia adapted mammals. Following this, I would search for orthologues in human genome. Finally, I would focus on these genes on all patients admitted to the intensive care unit. The aim would be to compare survivors and non-survivors to assess if these

specific genes are differently expressed.

A metobolomics approach has to be performed along with the gene expression study to ensure we have link between the gene expression and its functional correlate.

Leading on from this thesis, Prof Peters is due to start a feasibility study for a randomised control trial (GOSH Charity grant). The OXY-PICU is a feasibility study intended to inform a future randomised control trial of liberal vs. restrictive oxygen target. It is set out to investigate if:

1. Oxygen saturation targets of 88-92% and ≥95% are achievable in PICU patients.
2. It is possible to recruit the required number of patients.
3. It is safe for children to remain in the lower oxygen saturation category.

My systematic review, cohort study and the national survey set up the background for the OXY-PICU study. The review and cohort study demonstrated a U shaped relationship between PaO2 and mortality. The survey established the variability in oxygen administration across the PICUs in UK. In addition, it highlighted the equipoise in oxygen saturation targets for critically ill children (section 3.6).

Appendix A

Survey of oxygen targets used in paediatric intensive care units across the United Kingdom

Survey of oxygen targets used in paediatric intensive care units across the United Kingdom We are an intensive care team working in Great Ormond Street Hospital, London, UK. Although there is some evidence on the detrimental effects of hyperoxia, we think there may be an equipoise on the accepted oxygen delivery targets.

We would like to survey the prevelant practice across the ICUs. The survey should

take no more than 5 minutes. Thank you for taking the time to fill out this survey.

A.1 Characteristics of ICU and respondent

1. How many admissions does your ICU have in a year?

a. < 500

b. 500 - 1000

c. 1000 - 1500

d. > 1500

2. Is your ICU a cardio-surgical center?

a. Yes

b. No

3. Is your ICU a neuro-surgical center?

a. Yes

b. No

4. Age of respondent

a. 20 - 30 years

b. 31 - 40 years

c. 41 - 50 years

d. $>$ 50 years

5. Grade of person filling the survey

a. Consultant

b. Senior nurse

c. Junior nurse

d. Senior fellow

e. Junior fellow

6. Number of years of practice in intensive care

a. $<$ 2 years

b. 2 - 5 years

c. 5 - 10 years

d. $>$ 10 years

7. Do you have alarm targets on your oxygen saturation monitors?

a. Yes

b. No

c. Do not know

8. Do you have an oxygen weaning protocol for mechanically ventilated patients in your unit?

a. Yes

b. No

c. Do not know

A.2 Clinical scenario

A 1 year old male infant is mechanically ventilated. He has no premorbid conditions.

R/S:

BIPAP/ASB: PIP = 28 cm H2O, PEEP = 6 cm H_2O, ASB = 18 cm H_2O, Rate = 20 breaths per min, $FiO_2 = 0.8$, Saturations (Pulse oxymetry) = 94%.

CVS: HR = 125 beats per min, Art BP = 85/56 mm Hg, MBP = 66.

Neurology: Pupils - 3 mm, bilaterally equal and reactive, Morphine = 20 mcgs/kg/hr, Midazolam = 2 mcgs/kg/min. Not paralysed.

Arterial blood gas: pH: 7.32, PCO_2: 6.2, PaO_2: 10, BE: -ve 4, Lactate: 1.5.

Cardiac output- 4.6 l/min.

PIM2 predicted risk of mortality: 8.8

He has been ventilated for 2 days.

9. What are your PaO_2 targets for this patient?

a. $<$ 8 kPa

b. 8.1 - 10 kPa

c. 10.2 - 13 kPa

d. $>$ 13 kPa

e. I do not follow PaO_2 targets

10. Which combination of PaO_2 and FiO_2 targets would be more acceptable to you if he was admitted with Acute respiratory distress syndrome? (You may choose more than one combination)

Table A.1: Oxygen delivery in Acute respiratory distress syndrome

	$PaO_2 < 8$ kpa	$PaO_2 = 8.1$ to 10 kpa	$PaO_2 = 10.1$ to 13 kpa	$PaO_2 > 13$ kpa	I do not follow PaO_2 targets
FiO_2= 0.21					
$FiO_2 = 0.22$ to 0.4					
$FiO_2 = 0.41$ to 0.6					
$FiO_2 = 0.61$ to 0.8					
$FiO_2 = 0.81$ to 1					
I do not follow FiO_2 targets					

11. Which combination of PaO_2 and FiO_2 targets would be more acceptable to you if he was admitted following a Traumatic brain injury? (You may choose more than one combination)

Table A.2: Oxygen delivery in Traumatic brain injury

	$PaO_2 < 8$ kpa	$PaO_2 = 8.1$ to 10 kpa	$PaO_2 = 10.1$ to 13 kpa	$PaO_2 > 13$ kpa	I do not follow PaO_2 targets
$FiO_2 = 0.21$					
$FiO_2 = 0.22$ to 0.4					
$FiO_2 = 0.41$ to 0.6					
$FiO_2 = 0.61$ to 0.8					
$FiO_2 = 0.81$ to 1					
I do not follow FiO_2 targets					

12. Which combination of PaO_2 and FiO_2 targets would be more acceptable to you if he was admitted

Post cardiac arrest? (You may choose more than one combination)

Table A.3: Oxygen delivery in Post cardiac arrest

	$PaO_2 < 8$ kpa	$PaO_2 = 8.1$ to 10 kpa	$PaO_2 = 10.1$ to 13 kpa	$PaO_2 > 13$ kpa	I do not follow PaO_2 targets
$FiO_2 = 0.21$					
$FiO_2 = 0.22$ to 0.4					
$FiO_2 = 0.41$ to 0.6					
$FiO_2 = 0.61$ to 0.8					
$FiO_2 = 0.81$ to 1					
I do not follow FiO_2 targets					

13. Which combination of PaO_2 and FiO_2 targets would be more acceptable to you if he was admitted with Sepsis associated multi-organ failure? (You may choose more than one combination)

Table A.4: Oxygen delivery in Sepsis associated multi-organ failure

	PaO_2 < 8 kpa	PaO_2 = 8.1 to 10 kpa	PaO_2 = 10.1 to 13 kpa	PaO_2 > 13 kpa	I do not follow PaO_2 targets
FiO_2= 0.21					
FiO_2 = 0.22 to 0.4					
FiO_2 = 0.41 to 0.6					
FiO_2 = 0.61 to 0.8					
FiO_2 = 0.81 to 1					
I do not follow FiO_2 targets					

14. Which combination of PaO_2 and FiO_2 targets would be more acceptable to you if he was admitted with Pulmonary hypertension? (You may choose more than one combination)

Table A.5: Oxygen delivery in Pulmonary hypertension

	PaO_2 < 8 kpa	PaO_2 = 8.1 to 10 kpa	PaO_2 = 10.1 to 13 kpa	PaO_2 > 13 kpa	I do not follow PaO_2 targets
FiO_2 = 0.21					
FiO_2 = 0.22 to 0.4					
FiO_2 = 0.41 to 0.6					
FiO_2 = 0.61 to 0.8					
FiO_2 = 0.81 to 1					
I do not follow FiO_2 targets					

A.3 Further progress:

The child does not improve despite instituting and continuing intensive care for 24 hours. You have not been able to wean ventilation. The blood gases remain similar to those on admission. There is no change in the CXR.

15. Would you consider lowering the PaO_2 targets now?

Table A.6: Amended PaO_2 targets

	Yes	No	I do not follow PaO_2 targets
Acute respiratory distress syndrome			
Traumatic brain injury			
Sepsis associated multi-organ failure			
Post cardiac arrest			
Pulmonary hypertension			

16. What FiO_2 range would you accept to achieve your amended PaO_2 targets?

a. 0.21

b. 0.21 - 0.4

c. 0.41 - 0. 6

d. 0.61 - 0.8

e. 0.81 - 1

f. I will not amend my PaO_2 targets

g. I do not follow PaO_2 targets

A.4 Randomised control trial:

17. Is it ethical to perform a RCT with tight arterial oxygenation in critically ill children?

a. Yes

b. No

c. Maybe

d. Do not know

18. Do you believe that there is a need for a RCT aiming for tight arterial oxygenation?

a. Yes

b. No

c. Maybe

d. Do not know

19. Comments

Appendix B

Systematic review

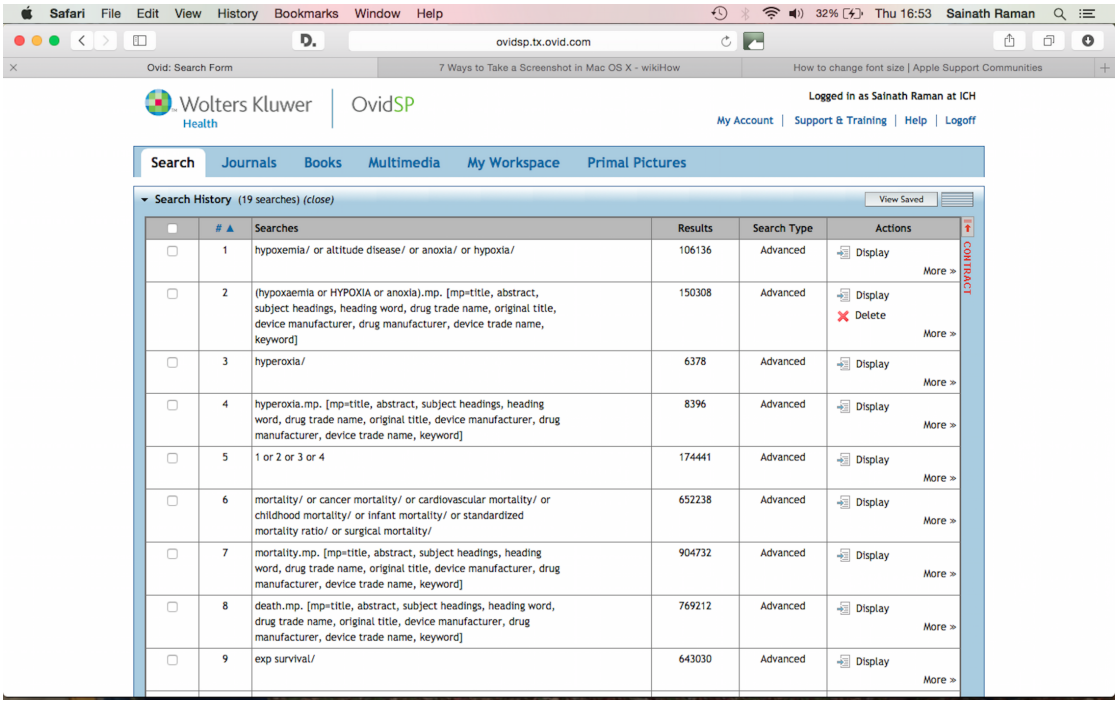
B.1 MEDLINE search strategy

("hypoxaemia"[All Fields] OR "hypoxia"[All Fields] OR "anoxia"[MeSH Terms] OR "Hyperoxia"[Mesh] OR "anoxia"[All Fields] OR "hypoxemia"[All Fields] OR "hyperoxia"[All Fields] OR "hyperoxaemia"[All Fields] OR "hyperoxemia"[All Fields]) AND ("mortality"[Subheading] OR "mortality"[All Fields] OR "morbidity"[All Fields] OR "morbidity"[MeSH Terms] OR "survival"[All Fields] OR "survival"[MeSH Terms]) NOT noninvasive[All Fields] NOT preterm[All Fields] NOT ("surface-active agents"[Pharmacological Action] OR "pulmonary surfactants"[Pharmacological Action] OR "surface-active agents" [MeSH Terms] OR ("surface-active"[All Fields] AND "agents"[All Fields]) OR "surface-active agents"[All Fields] OR "surfac-

tant"[All Fields] OR "pulmonary surfactants"[MeSH Terms] OR ("pulmonary"[All Fields] AND "surfactants"[All Fields]) OR "pulmonary surfactants"[All Fields]) AND (("1950/01/01"[PDAT] : "2015/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms] OR "infant"[All Fields] AND "newborn"[All Fields]) OR "newborn infant"[All Fields] OR "neonates"[All Fields]) AND "humans"[MeSH Terms] NOT ("hypoxic ischaemic encephalopathy"[All Fields] OR "hypoxia-ischemia, brain"[MeSH Terms] OR ("hypoxia-ischemia"[All Fields] AND "brain"[All Fields]) OR "brain hypoxia-ischemia" [All Fields] OR ("hypoxic"[All Fields] AND "ischemic"[All Fields] AND "encephalopathy"[All Fields]) OR "hypoxic ischemic encephalopathy"[All Fields]) NOT perinatal[All Fields] AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]) AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]) NOT ("adult"[MeSH Terms] OR "adult"[All Fields]) AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]) NOT ("extracorporeal membrane oxygenation"[MeSH Terms] OR ("extracorporeal"[All Fields] AND "membrane"[All Fields] AND "oxygenation"[All Fields]) OR "extracorporeal membrane oxygenation"[All Fields] OR "ecmo"[All Fields]).

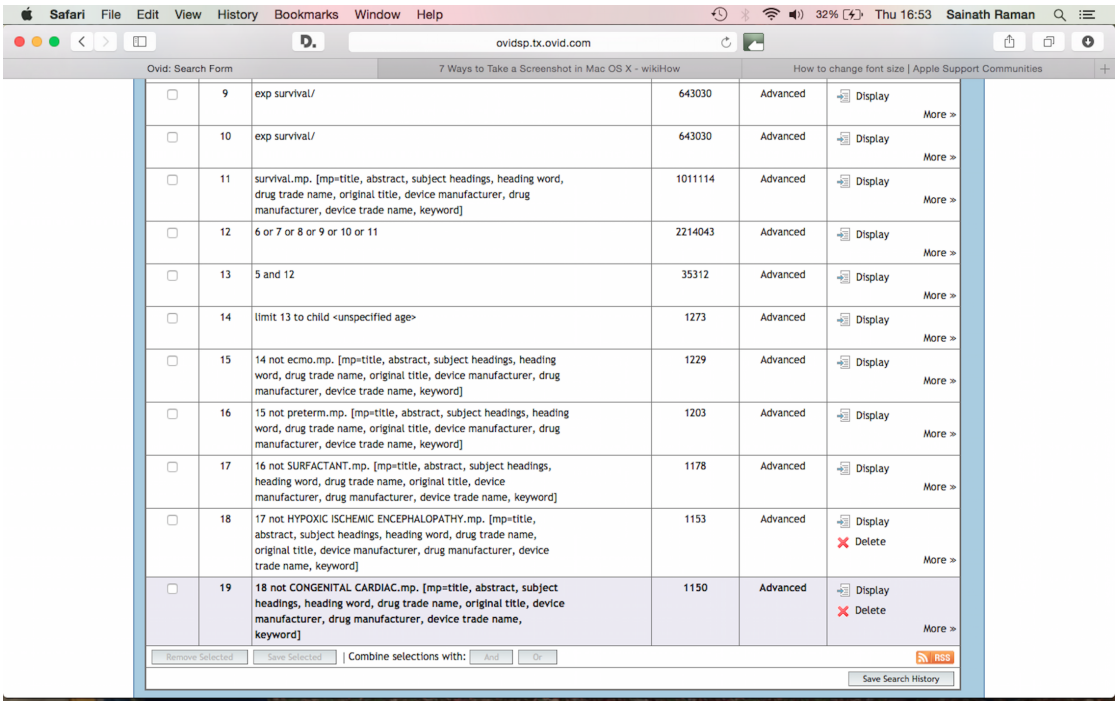
B.2 EMBASE search strategy

Figure B.1: Snapshot of Embase search for the systematic review



	# ▲	Searches	Results	Search Type	Actions
☐	1	hypoxemia/ or altitude disease/ or anoxia/ or hypoxia/	106136	Advanced	Display More »
☐	2	(hypoxaemia or HYPOXIA or anoxia).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	150308	Advanced	Display Delete More »
☐	3	hyperoxia/	6378	Advanced	Display More »
☐	4	hyperoxia.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	8396	Advanced	Display More »
☐	5	1 or 2 or 3 or 4	174441	Advanced	Display More »
☐	6	mortality/ or cancer mortality/ or cardiovascular mortality/ or childhood mortality/ or infant mortality/ or standardized mortality ratio/ or surgical mortality/	652238	Advanced	Display More »
☐	7	mortality.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	904732	Advanced	Display More »
☐	8	death.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	769212	Advanced	Display More »
☐	9	exp survival/	643030	Advanced	Display More »

Figure B.2: Snapshot of Embase search for the systematic review



Appendix C

Respiratory Electron Transport Chain gene set

Figure C.1: Enrichment plot of Respiratory Electron Transport Chain gene set

GENE SYMBOL	GENE TITLE	Rank in gene list	Rank metric score	Running ES
NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	141	0.580737412	0.025490472
NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	142	0.579333186	0.057739805
NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa	151	0.575001955	0.08936012
NDUFA9	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9, 39kDa	244	0.544995785	0.115236975
CYCS	cytochrome c, somatic	322	0.524983287	0.14072716
ETFDH	electron-transferring-flavoprotein dehydrogenase	389	0.511933982	0.16602431
NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9kDa	395	0.510708988	0.19421114
ATP5F1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit B1	459	0.500089526	0.21899444
ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	627	0.474581122	0.23731485
COX6C	cytochrome c oxidase subunit VIc	629	0.474380046	0.26367334
ETFA	electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)	849	0.444986284	0.27782488
ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	1057	0.418873042	0.29110464
NDUFB4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa	1059	0.41816777	0.314334
NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa	1067	0.41703403	0.3372093
UQCRB	ubiquinol-cytochrome c reductase binding protein	1068	0.416952997	0.36041954
ATP5H	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	1078	0.416566819	0.3831719
UQCRC2	ubiquinol-cytochrome c reductase core protein II	1204	0.407581508	0.39979923
COX4I1	cytochrome c oxidase subunit IV isoform 1	1361	0.39220345	0.4140674
ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	1457	0.383250266	0.43079498
NDUFA12	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12	1508	0.378065348	0.449416
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	1509	0.377965599	0.47005364

Figure C.2: Enrichment plot of Respiratory Electron Transport Chain gene set

NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa	1802	0.352161109	0.4758671
ATP5L	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G	1948	0.339935333	0.48775902
NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)	1977	0.33788529	0.50521016
NDUFB3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa	2022	0.33430174	0.52168596
COX7B	cytochrome c oxidase subunit VIIb	2038	0.333067089	0.5394992
ATP5J	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6	2054	0.331590444	0.5572303
COX7C	cytochrome c oxidase subunit VIIc	2078	0.330410659	0.5745078
ATP5O	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein)	2432	0.304306418	0.57433057
COX5A	cytochrome c oxidase subunit Va	2910	0.274319977	0.56647146
ATP5G1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C1 (subunit 9)	3502	0.235096127	0.55090106
UQCRH	ubiquinol-cytochrome c reductase hinge protein	3536	0.233377218	0.5622921
NDUFC1	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa	3565	0.232144445	0.57385707
UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	3573	0.231671199	0.5864139
COX8A	cytochrome c oxidase subunit 8A (ubiquitous)	3857	0.216539413	0.5847453
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	4017	0.20855625	0.588645
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)	4055	0.206166953	0.59832746
COX5B	cytochrome c oxidase subunit Vb	4070	0.204730973	0.6090452
COX7A2L	cytochrome c oxidase subunit VIIa polypeptide 2 like	4793	0.164833248	0.5832114
ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	4925	0.158635601	0.58568996
NDUFS6	NADH dehydrogenase (ubiquinone) Fe-S protein 6, 13kDa (NADH-coenzyme Q reductase)	5084	0.151797757	0.5864786
NDUFV3	NADH dehydrogenase (ubiquinone) flavoprotein 3, 10kDa	5317	0.139424875	0.5829903
SDHA	succinate dehydrogenase complex,	5563	0.12870793	0.578275

Legend: RETC gene set2.

Figure C.3: Enrichment plot of Respiratory Electron Transport Chain gene set

	subunit A, flavoprotein (Fp)			
NDUFB10	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22kDa	5966	0.112232514	0.5650298
COX6B1	cytochrome c oxidase subunit Vib polypeptide 1 (ubiquitous)	6021	0.109510086	0.56850743
CYC1	cytochrome c-1	6168	0.10344518	0.56718636
NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase)	6233	0.100754522	0.56969166
NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa	6476	0.090301804	0.56298393
NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa	6788	0.078439519	0.5522701
COX6A1	cytochrome c oxidase subunit VIa polypeptide 1	6992	0.071207188	0.5463906
ETFB	electron-transfer-flavoprotein, beta polypeptide	7028	0.070103228	0.54859585
NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)	7045	0.069131777	0.5516683
NDUFS8	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)	7226	0.061985802	0.5463907
NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa	7583	0.04997398	0.5319103
NDUFA11	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 11, 14.7kDa	7793	0.043314729	0.52418715
UCP3	uncoupling protein 3 (mitochondrial, proton carrier)	7874	0.040199824	0.52254575
NDUFC2	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2, 14.5kDa	8420	0.023468345	0.49742535
NDUFA3	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3, 9kDa	9388	-0.004821032	0.45080432
NDUFA7	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7, 14.5kDa	10547	-0.037801318	0.3967577
UCP1	uncoupling protein 1 (mitochondrial, proton carrier)	10891	-0.047396649	0.38276416
NDUFS7	NADH dehydrogenase (ubiquinone) Fe-S protein 7, 20kDa (NADH-coenzyme Q reductase)	11286	-0.057582371	0.36686468
NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)	11531	-0.064274073	0.35861114
ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit	13420	-0.116965681	0.2735739

Legend: RETC gene set3.

Figure C.4: Enrichment plot of Respiratory Electron Transport Chain gene set

NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa	14687	-0.152922139	0.22069876
SDHB	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	15205	-0.168352574	0.20500122
SDHC	succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa	15255	-0.169820607	0.21207851
SDHD	succinate dehydrogenase complex, subunit D, integral membrane protein	15783	-0.186606452	0.19691221
NDUFB1	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 1, 7kDa	16512	-0.211896777	0.17340733
UQCRC1	ubiquinol-cytochrome c reductase core protein I	17390	-0.24948962	0.14477016
NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17kDa	17911	-0.274745822	0.13484967

Legend: RETC gene set4.

Appendix D

Functional data analysis of the YES2 dataset

D.1 Fitting individual curve

D.1.1 *a. Smoothing spline approach (lambda specification, knots positioning, penalisation argument)*

1. Data:

We have NIRS VOT curves from 12 children at two altitudes (London and Namche). The range of our values will be:

rangeval = c(0.5,675.5) (1)

All 24 records with Time as rows and IDs as different columns were collated to create a CSV file. We use R software for this analysis. Changing the CSV file into a matrix and removing the Time column from it creates a matrix (toimatrix).

2. Basis splines:

Sequence of Knots The Knotvec

A vector containing the time points where we want knots to be placed is created. This vector starts at 0.5 and ends at 675.5. The breaks are at 290.5 and 458. We will place knots at each observation time point. Consequently, we have a total of 1349 interior knots are placed.

Knotvec (breaks) = c(0.5,150, 290.5,290.5,290.5,375,458,458,458,475,500,525,550,575,600,625,650,675

(2)

Order

Ours is a cubic spline, hence of degree 3 and order 4 (norder).

Number of basis functions (nbasis) = Order + Number of interior knots

nbasis = 4 +16 = 20

Creating the basis system

basisspline = create.bspline.basis(rangeval, nbasis, norder, breaks)

basisspline = create.bspline.basis(c(0.5,675.5,15,4,knotvec) (3)

3. Creating functional curves for our data

R creates smoothing curve by using regression analysis.

smooth.basis(argvals, y, fdParobj, wtvec=rep(1, length(argvals)), fdnames=NULL)

toicurve = smooth.basis(Time, toimatrix, basisspline) (4)

toifd = toicurve$fd

$toiy2cmap = toicurve$y2cmap

Roughness penalty

Roughness penalty needs to added because the smoothing function creates a curve that may be too smooth and overfitted to the data. To define roughness penalty, we need to construct a functional parameter object. We need to define three parameters.

1. Basis object: In our case, this would be the basisspline we created earlier (equation 3).

2. The derivative of order m (or linear differential operator) to be penalized: Order 2 in for our data.

3. A smoothing parameter (lambda): For now, we will set lambda at 0.1. We will discuss this later.

So the equation becomes:

toifdPar = fdPar(basisspline, 2, $10^{0.25}$) (5)

Now, we use the fdpar object in the equation 4 and change it to:

toicurve = smooth.basis(Time, toimatrix, toifdPar) (6)

To create a functional data object from within this function, we use $fd.

toicurvefd = toicurve$fd

toicurveSSE = toicurve$SSE

toicurvedf = toicurve$df

toicurvey2cmap = toicurve$y2cmap

Choosing a smoothing parameter

A vector of generalized cross-validated measure (GCV) for $Log_{10}lambda$ values -18 to 9 was created. The lowest GCV was at 0.25.
Hence, was set at $10^{0.25}$. This is the value used in equation (5). With the above equations, the predicted curves for all 12 children at both the altitudes are produced.

Labelling functional data objects

We need three labels. A label for domain (Time), a label for replication dimension

(Child) and a label for the range (Tissue oxygen index TOI)

fdnames = vector("list", 3)

fdnames[[1]] = "Time (secs)"

fdnames[[2]] = "Child"

fdnames[[3]] = "Tissue oxygen index (TOI)"

Child = vector("list", 12)

Child[[1]] = "ID1"

.

.

.

Child[[12]] = "ID12"

D.1.2 Series of figures showing the original points and the fitted curve with a measure of fit.

2. Estimate the effect of altitude with FANOVA

a. Coefficient function with its confidence intervals for the two places

I set the altitude as the exposure and compare the two altitudes. By considering the fitted curves as a regression, we get coefficients for each of the curves (equation 7).

sample.coef = toifd$coef (7)

We have to augment the columns by one (equation 8).

sample.coef.aug = cbind(sample.coef,matrix(0,1353,1)) (8)

toi.aug = fd(sample.coef.aug, splinebasisall) (9)

predic.toi.aug = eval.fd(table1$Time,toi.aug) (10)

fRegressionaltitude = fRegress(toi.aug, altitudelist, betalist) (11)

The altitudelist is a vector of the two altitudes.

coefnum = eval.fd(Time, betaestlistaltitude[[2]]$fd) (12)

Equation 12 gives us the coefficients for the two altitudes. At the lowest point of TOI, it is 1.2957.

b. Predicted curves in the two altitudes

altitudefit = fRegressionaltitude$yhatfd (13)

c. Functional F test

F.resaltitude = Fperm.fd(toi.aug, altitudelist, betalist) (14)

Fstatisticsaltitude = F.resaltitude$Fobs (15)

The Fstatisticaltitude value is 19.97127.

ninetyfivethquartilealtitude = F.resaltitude$qval (16)

The ninetyfivethquartilealtitude value is 0.2427882.

D.1.3 3. Functional Data Analysis with Mixed effects

x = Time

y = table1a[,2]

for (i in 2:24)

y = c(y,table1a[,i+1])

test.frame = data.frame(y,x,id,sample,Sex, Altitude) (17)

Altitude0 = as.factor(table5$Altitude)

Sex0 = as.factor(table5$Sex)

sample0 = as.factor(c(1:12,1:12))

id0 = as.factor(1:24)

est = fdaLm(y—id0 Altitude0+Sex0—0+sample0,data=test.frame) (18)

est$betaHat

est$betaVar

```
est$Ghat

est$uBLUP

plot(x,est$betaHat[,"Altitude01"])

matplot(x, cbind(est$betaHat[,"Altitude01"],

est$betaHat[,"Altitude01"] + 2*(sqrt(est$betaVar[2,2])),

est$betaHat[,"Altitude01"] - 2*(sqrt(est$betaVar[2,2]))), type="l",lty=c(1,4,4), xlab="Time",

ylab="Reg.Coeff.")


summary(est) (19)
```

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Research Article

Survey of Oxygen Delivery Practices in UK Paediatric Intensive Care Units

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Purpose. Administration of supplemental oxygen is common in paediatric intensive care. We explored the current practice of oxygen administration using a case vignette in paediatric intensive care units (PICU) in the united kingdom. *Methods.* We conducted an online survey of Paediatric Intensive Care Society members in the UK. The survey outlined a clinical scenario followed by questions on oxygenation targets for 5 common diagnoses seen in critically ill children. *Results.* Fifty-three paediatric intensive care unit members from 10 institutions completed the survey. In a child with moderate ventilatory requirements, 21 respondents (42%) did not follow arterial partial pressure of oxygen (PaO_2) targets. In acute respiratory distress syndrome, cardiac arrest, and sepsis, there was a trend to aim for lower PaO_2 as the fraction of inspired oxygen (FiO_2) increased. Conversely, in traumatic brain injury and pulmonary hypertension, respondents aimed for normal PaO_2 even as the FiO_2 increased. *Conclusions.* In this sample of clinicians PaO_2 targets were not commonly used. Clinicians target lower PaO_2 as FiO_2 increases in acute respiratory distress syndrome, cardiac arrest, and sepsis whilst targeting normal range irrespective of FiO_2 in traumatic brain injury and pulmonary hypertension.

1. Introduction

The administration of supplemental oxygen is common in the critically ill. The aim is to augment oxygen delivery to the tissues [1]. Hyperoxia can lead to production of reactive oxygen species and cell injury [2, 3]. Conversely, hypoxia causes cell death. The "ideal" PaO_2 target range is unclear. Consequently clinical practice varies.

Eastwood et al. reported that 77% of intensivists in Australia and New Zealand prescribed oxygen saturation targets. Clinicians working in regional centers were less concerned with oxygen toxicity [4]. De Graaff et al. explored the response of Dutch clinicians to arterial blood gas values (ABG) in tertiary intensive care units. The FiO_2 was reduced in only 25% of situations with a $PaO_2 > 16$ kPa [5].

The etiology and evolution of paediatric critical illness are different to adults. Multiorgan failure (MOF) occurs early in children and they have better survival [6, 7]. Nonetheless, the duration of mechanical ventilation and length of stay in the paediatric intensive care unit is increasing [8]. This survey aimed to describe prevalent paediatric intensive care practice, existence of weaning protocols, and if a clinical equipoise exists between liberal and restrictive oxygenation targets.

2. Material and Methods

All the members of the Paediatric Intensive Care Society (PICS), UK, were requested to complete an online survey. PICS consists of nursing, medical, and allied health professionals working in paediatric intensive care units. The practitioners from the neonatal intensive care units in UK were not approached, as their patient profile is significantly different.

The survey was designed by the authors and published using a survey website (https://opinio.ucl.ac.uk). Demographic data including age, ICU type, their seniority, and years of practice were sought. The study was discussed with the chair of Bloomsbury Research and Ethics Committee (London, UK). We were advised that a formal ethics review was not required.

Table 1: Characteristics of respondents.

Number of admissions/year to your intensive care unit	Number (%)
<500	9 (17.6)
501–1000	24 (47)
1001–1500	13 (25.5)
>1500	5 (9.8)
No response	2
Cardiosurgical center	33 (66)
Neurosurgical center	35 (67)
Grade of respondent	
Consultant	25 (48)
Senior nurse	10 (19.2)
Senior fellow	12 (23)
Junior fellow	5 (9.6)
No response	1
Number of years of practice in intensive care	
2–5 years	13 (25.5)
5-6 years	13 (25.5)
>10 years	25 (49)
No response	2

The survey outlined the following clinical scenario: a 1-year-old patient with no premorbid conditions is ventilated with peak inspiratory pressure of 28 cm H_2O, positive end expiratory pressure of 6 cm H_2O, respiratory rate of 20 breaths per min, and FiO_2 of 0.8. His peripheral oxygen saturation (pulse oximetry), heart rate, blood pressure, and mean blood pressure are 94%, 125 beats per min, 85/56 mmHg, and 66 mmHg, respectively. He has bilaterally equal and reactive pupils measuring 3 mm. He is sedated on intravenous morphine and midazolam. He is not paralysed. Latest arterial blood gas values are as follows: pH: 7.32, PCO_2: 6.2 kPa, PaO_2: 10 kPa, BE: -ve 4, and lactate: 1.5 mmol/L. The PIM2 predicted risk of mortality is 8.8%. He has been ventilated for 2 days.

With the same clinical history, clinicians were asked to decide on the oxygenation targets when the potential diagnosis is ARDS, CA, Sepsis, TBI, or PHTN.

A further question explored if weaning protocols were in place in their units. The need for a randomised control trial (RCT) with tight arterial oxygenation targets was explored.

3. Results

Only 30% (53) of those whom were invited to participate in the online survey responded. The majority of respondents worked in moderate sized ICUs, with admission rates between 500 and 1000 patients per annum. The characteristics of the respondents are presented in Table 1.

The majority of units (96%) had an alarm target on their oxygen saturation monitor. Thirty-eight respondents (73%) worked in units that did not have an oxygen weaning protocol for mechanically ventilated patients. The units with admissions more than 1500 were less likely to have a weaning protocol compared to those between 500 and 1500 admissions.

For the given clinical scenario, 21 respondents (42%) did not follow PaO_2 targets. Of the rest, 21 clinicians (42%) targeted PaO_2 between 8.1 and 10 kPa. Only 8 (16%) aimed for the normal range (10.1–13 kPa).

In ARDS, CA, and sepsis, there was a tendency to aim for lower PaO_2 (<10 kPa) as the FiO_2 increased. This was noticeable when the FiO_2 was more than 0.4 (45%) which equates to a PaO_2/FiO_2 ratio of less than 200. Following TBI and in PHTN, there was a propensity to aim for normal PaO_2 (10.1–13 kPa) even as the FiO_2 rose (28–33% when FiO_2 > 0.4). In TBI, the proportion of respondents targeting a lower PaO_2 increased when the FiO_2 was more than 0.8 (8%). A proportion of respondents targeted PaO_2 ranges above normal (15%). In PHTN, normal range remained the preferred range throughout the range of FiO_2 (Figure 1).

The initial scenario was further extended as "no improvement after 24 hours of intensive care." The management strategy did not change in this setting.

Thirty-nine percent considered it ethical to conduct a RCT with tight arterial oxygenation target whilst 11% did not. The remaining respondents were undecided.

4. Discussion

Our survey shows that, practice variation notwithstanding, there seems to be a general consensus to aim for lower PaO_2 in the setting of ARDS, CA, and sepsis. The results are consistent with higher PaO_2 targets being chosen in children following TBI and in PHTN. Only a small proportion of respondents felt a RCT with tight oxygenation target would be unethical.

Paediatric intensivists tolerate a low SpO_2 target (88%) with a low tidal volume strategy for ARDS [9]. Our findings concur. A recent point prevalence study reported that adult intensive care practitioners aim to prevent low oxygen saturation (SpO_2 < 90%) but fail to address high saturations [10]. This is in the face of mounting evidence of harm from hyperoxia [11]. Should we aim for a restrictive oxygenation target in critically ill patients? The "HOT or NOT" trial showed that separation between titrated oxygen target and standard target is possible in intensive care [12]. A recent multicenter study demonstrated no difference in 90-day mortality between mechanically ventilated patients randomised to a conservative (pulse oximetry: 88–92%) and liberal oxygenation targets (>96%) [13]. A larger randomised control trial is awaited.

The main limitation of this survey is the likely low response rate. At the time of the survey the membership of the society was not well defined. Responses were not sought beyond a single e-mail. The low number of respondents from junior staff perhaps suggests that considerable experience is needed to set distinct targets in these clinical scenarios. Despite this limitation, the results indicate that restrictive targets are aimed for in certain scenarios.

We had intended to analyse Cohen's kappa to look at interrater agreement. However, due to the small sample size a formal statistical analysis was not attempted.

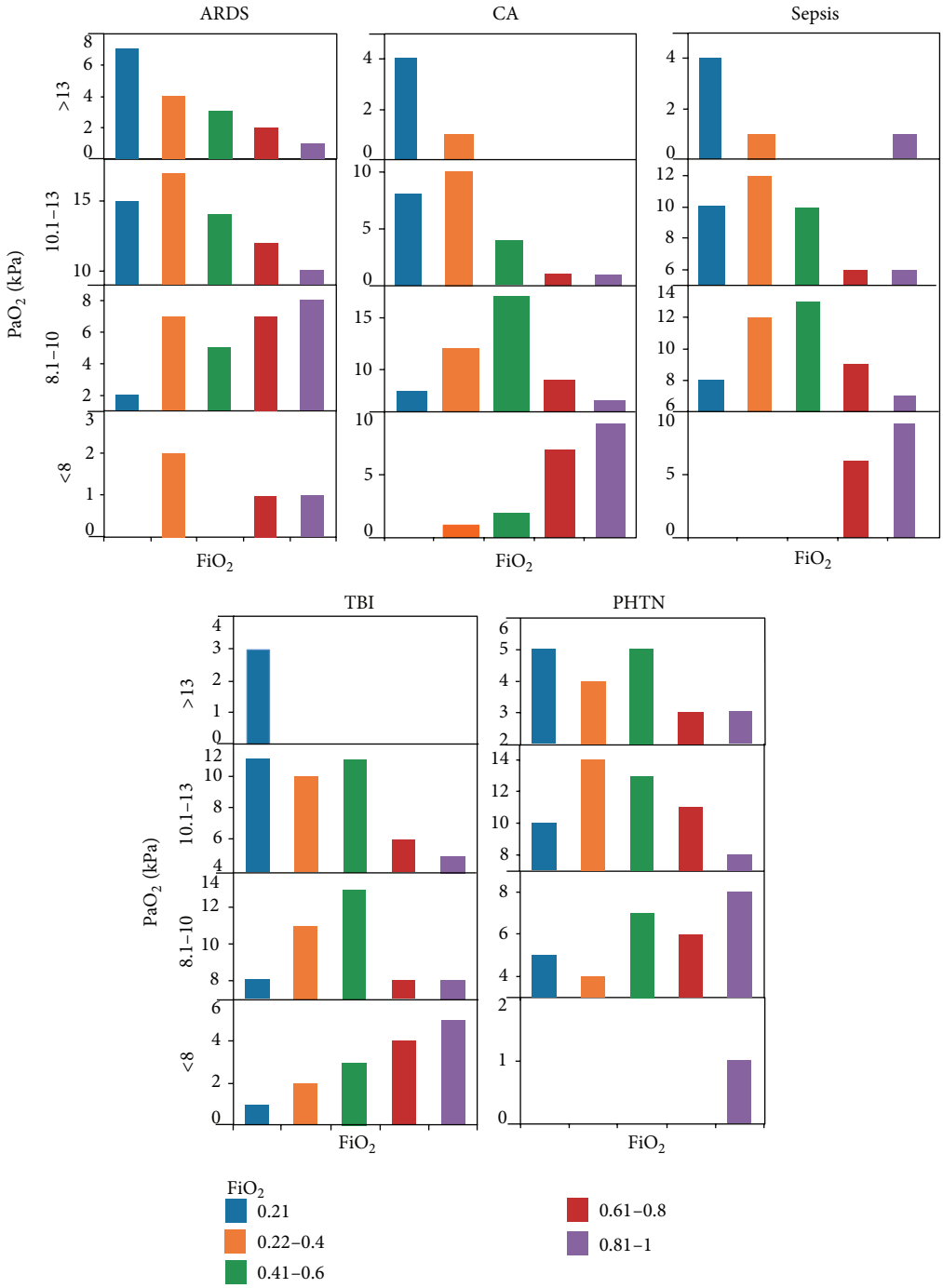

Figure 1: The profile of PaO_2 (y-axis) and FiO_2 (x-axis) targeted in 5 clinical scenarios in a child with moderate ventilatory requirements. The PaO_2 ranges from <8 kPa in the bottom panel to >13 kPa in the top panel within each scenario. FiO_2 ranges from 0.21 (blue) through to 0.81–1 (purple). The three scenarios in the upper section show a pattern of more restrictive PaO_2 targets with increasing FiO_2. The 2 scenarios in the lower section show that higher normal PaO_2 ranges are targeted irrespective of increasing FiO_2.

5. Conclusions

In this study variability and lack of consensus are consistent with an assumption of clinical equipoise. Supplemental oxygen administration practices and oxygenation target practices vary. A majority of respondents worked in units with no oxygen weaning protocol. A proportion of clinicians do not follow PaO_2 targets. Clinicians aim for lower PaO_2 thresholds in ARDS, CA, and sepsis whilst aiming for the normal range in TBI and PHTN. The lack of consensus and the large variability in practice demonstrate equipoise. This should be addressed with a feasibility trial comparing restrictive to

standard oxygenation targets in critically ill children to lead up to a future RCT.

Disclosure

Dr. Raman is a holder of HCA fellowship.

Competing Interests

The authors declare that they have no competing interests.

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LETTER

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Oxidative phosphorylation gene expression falls at onset and throughout the development of meningococcal sepsis-induced multi-organ failure in children

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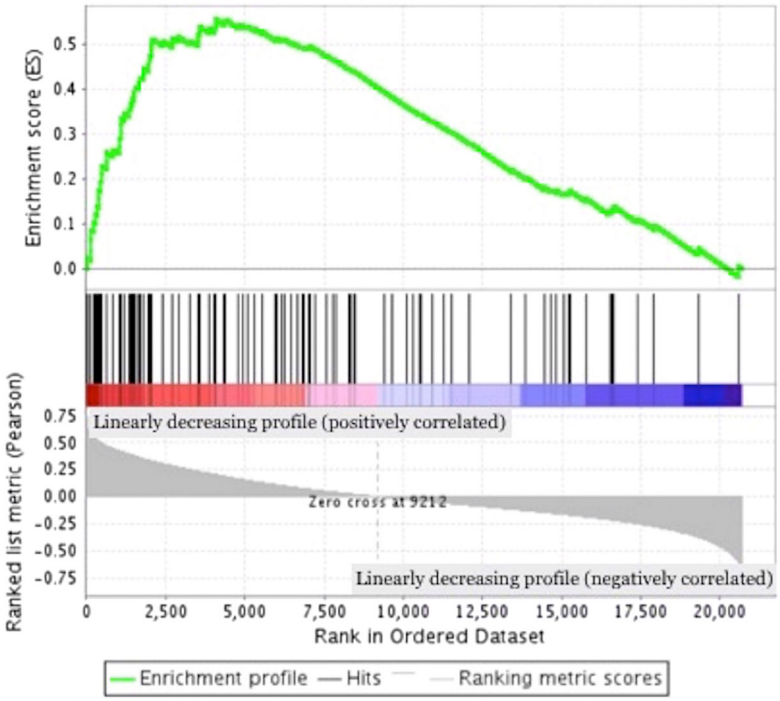
Dear Editor,

Sepsis-induced critical illness differs between adults and children. Children deteriorate more quickly and exhibit a 'cold shock' haemodynamic pattern more often. Organ failure scores characteristically peak earlier in septic children (<2 ICU days) than adults (days 3–4). A high proportion of deaths in children occur very early [1]. Yet, ICU stays are shorter and survival better in children [2]. Might these differences in clinical phenotype—more rapid onset and recovery—be due to differences in the underlying mechanisms of multi-organ failure (MOF)?

Acute mitochondrial dysfunction may contribute to MOF. Reduced mitochondrial oxygen utilisation and gene expression has been observed in established sepsis in adults and children [3]. There is an association between recovery of mitochondrial function and survival but the contribution to the onset of organ failure is less clear. We investigated whether mitochondrial oxidative phosphorylation gene expression (Oxphos) alters early enough in the clinical course of sepsis to remain a candidate element of MOF pathophysiology. To do this, we selected a population with the most rapid onset of profound sepsis-MOF: previously healthy children with acute meningococcal septicaemia. We investigated the time-course of gene expression in peripheral blood with gene set enrichment analysis (GSEA), at 0, 4, 8, 12, 24, and 48 h from time of admission to the emergency room. Extracted RNA was hybridised in Affymetrix microarrays. Methodological details are published elsewhere [4]. The dataset is available to download from the European Bioinformatics Institute database (ArrayExpress id: E-MEXP-3850). Emergency room venesection was designated time 0 as a pragmatic



Patient	NES	Nominal p value	FDR	RETC position	Gene sets with decreasing expression
Group	2.55	**<0.001**	0	75	1920

Fig. 1 Enrichment plot of the Respiratory Electron Transport Chain (RETC) gene set showing the normalised enrichment score (*NES*), the false discovery rate (*FDR*) and nominal *p* values for the gene set. Gene set enrichment analysis compares the variation in the gene expression in our overall dataset compared to a preselected gene set (RETC gene set) over the time-course. We have observed that RETC gene set expression is down-regulated more than would be expected by background variation in expression. The *top* portion of the plot shows the running enrichment score (*ES*) for the RETC gene set. The *middle* portion of the plot shows where the members of the gene set appear in the ranked list of genes. The *lower* portion of the plot shows the value of the ranking metric as you move down the list of ranked genes. The ranking metric measures a gene's correlation with a phenotype. For our continuous phenotype (time series), a *positive value* indicates correlation with the phenotype profile (decreasing profile)

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reference time point while acknowledging that children may be at different stages of illness at presentation.

The GSEA ranks changes in single gene expression. It notes the distribution of elements of a predefined gene set in this overall ranked list. We focused on the Reactome TCA cycle and respiratory electron transport chain (RETC) genes within the 3655 included gene sets. GSEA describes the probability of a non-random distribution of RTEC elements within the ranked list [5].

On the group level analysis, with a false discovery rate (FDR) at <25 % and ranked according to their normalised enrichment score (NES), 1039 out of 3655 gene sets showed a decreasing profile with time. The RETC set was ranked 75th of the 1039 gene sets.

On the individual-level analysis and FDR <25 %, all five patients had a highly ranked fall in RETC set expression. Patient 1 showed a low correlation of RETC gene expression to a decreasing profile. This might be relevant as, unfortunately, this child died. Figure 1 shows the NES, FDR and nominal p values for the RETC set for the individual patients and the overall group.

Oxidative phosphorylation gene expression reduced early and continued to decrease for at least 48 h in septic critically ill children. These findings are consistent with mitochondrial dysfunction contributing to the development of organ failure in both adults and children despite the differences in sepsis phenotype in these groups.

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Conflicts of interest The authors do not have any competing interests.

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